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BINAURAL MECHANISM REVEALED WITH IN VIVO WHOLE CELL PATCH CLAMP RECORDINGS IN THE INFERIOR COLLICULUS

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by

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Dedication

To my parents, my daughter and my husband

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BINAURAL MECHANISM REVEALED WITH IN VIVO WHOLE CELL PATCH CLAMP RECORDINGS IN THE INFERIOR COLLICULUS

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Many cells in the inferior colliculus (IC) are excited by contralateral and inhibited by ipsilateral stimulation and are thought to be important for sound localization. These excitatory-inhibitory (EI) cells comprise a diverse group, even though they exhibit a common binaural response property. Previous extracellular studies proposed specific excitatory and/or inhibitory events that should be evoked by each ear and thereby generate each of the EI discharge properties. The proposals were inferences based on the well established response features of neurons in lower nuclei, the projections of those nuclei, their excitatory or inhibitory neurochemistry, and the changes in response features that occurred when inhibition was blocked.

Here we recorded the inputs, the postsynaptic potentials, discharges evoked by monaural and binaural signals in EI cells with in vivo whole cell recordings from the inferior colliculus (IC) of awake bats. We also computed the excitatory and inhibitory synaptic conductances from the recorded sound evoked responses. First, we showed that a minority of EI cells either inherited their binaural property from a lower binaural nucleus or the EI property was created in the IC via inhibitory projections from the ipsilateral ear, features consistent with those observed in extracellular studies. Second, we showed that in a majority of EI cells ipsilateral signals evoked subthreshold EPSPs that behaved paradoxically in that EPSP amplitudes increased with intensity, even though binaural signals with the same ipsilateral intensities generated progressively greater spike suppressions. These ipsilateral EPSPs were unexpected since they could not have been detected with extracellular recordings. These additional responses suggested that the circuitry underlying EI cells was more complex than previously suggested. We also proposed the functional significance of ipsilaterally evoked EPSPs in responding to moving sound sources or multiple sounds. Third, by computing synaptic conductances, we showed the circuitry of the EI cells was even more complicated than those suggested by PSPs, and we also evaluated how the binaural property was produced by the contralateral and ipsilateral synaptic events.

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CHAPTER 1: *INTRODUCTION*

FREQUENCY REPRESENTATION IS THE FUNDAMENTAL ORGANIZING PRINCIPLE OF THE AUDITORY SYSTEM

The primary function of the sensory surface of the cochlea is to perform a frequency-to-place transformation of incoming sounds and therefore generate a map of frequency along the length of the basilar membrane, an organization referred as a tonotopy (Bekesy 1947, 1960). The map in all animals is that high frequencies are represented in the basal regions of the basilar membrane and low frequencies are represented in progressively more apical regions. Each auditory nerve fiber innervates one point on the cochlea and thus is maximally sensitive to the frequency that corresponds to that location on the basilar membrane (Kiang, Pfeiffer et al. 1965). This feature is maintained so the population of auditory nerve fibers recreates the tonotopy of the cochlea. The projections of the auditory nerve fibers whose innervation is from one place on the basilar membrane then terminate on sheets of cells in cochlear nucleus which the first synaptic station of the central auditory system (van Noort 1969). Therefore the sheet of cells receiving projections from those auditory nerve fibers are all turned to the same frequency and are referred to as isofrequency. Each sheet then forms an isofrequency contour within the nucleus, with adjacent sheets representing adjacent

portions of the basilar membrane. Thus the tonotopy of the basilar membrane is reproduced as an orderly succession of isofrequency contours that comprise the cochlear nucleus. The neurons in each isofrequency contour of the cochlear nucleus then project to corresponding isofrequency contours in higher nuclei, the superior olivary nuclei, and through the ascending auditory system, from cochlea nucleus to auditory cortex (Fig 1.1).

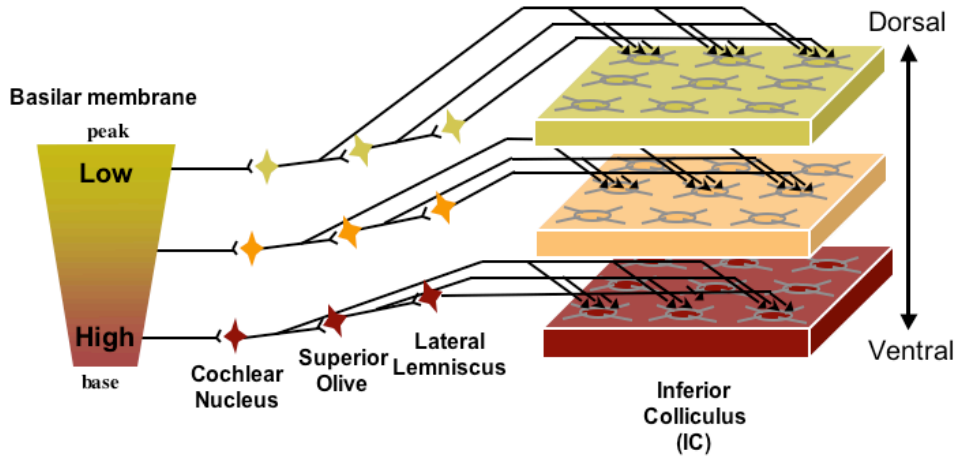

Fig 1.1. Tonotopic organization of the auditory system. Low frequencies are represented in the peak, and high frequency are represented in the base part of the basilar membrane. Projections from a location along the basilar membrane innervate a sheet of cells in cochlear nucleus (CN), forming an isofrequency contour. The adjacent sheets represent adjacent portions of the basilar membrane. The tonotopy of the basilar membrane is reproduced as an orderly succession of isofrequency contours that comprise the cochlear nucleus. The neurons in each isofrequency contour of the cochlear nucleus then project to corresponding isofrequency contours in higher nuclei, the superior olivary nuclei, lateral lemniscus, and inferior colliculus (IC) and through the ascending auditory system.

IIDs ARE THE CUES FOR LOCALIZING HIGH FREQUENCY SOUND SIGNAL

Since the cochlea maps frequency, the information about the location of a sound source is not present on the cochlear surface. Rather the auditory system has to make computations to localize a sound. The computation is to compare the arrival time or intensity of a sound received at one ear with the same features received by the other ear. Thus the ability to determine the location of a sound depends on binaural processing.

Low frequency sounds are localized by comparing the arrival times (ITDs) of sound received at two ears. Low frequencies are not blocked by the head and ears, but rather bend around the head. As a consequence, sounds that emanate from locations on one side do not produce differences in intensity at two ears. Rather, a sound off the midline takes a longer time to reach one ear than the other due to the difference in path length they have to travel to reach each ear. The farther to one side that the sound source is located, the longer is the path length to the farther ear, and hence the greater the ITD. Thus, the information about the azimuthal location of low frequencies is contained in their ITDs (Jeffress 1948; Konishi 1973; Heffner and Heffner 1988; Stern, Zeiberg et al. 1988; Carr and Konishi 1990).

All animals, including echolocating bats, use interaural intensity disparities (IIDs) to localize high frequency sounds (Erulkar 1972; Erulkar 1972; Mills 1972). IIDs are generated by two mechanisms. One is the acoustic barrier caused by head and ears. Therefore a sound of a given frequency located on one side of head is blocked to a lesser

degree on that side and to a greater degree on the other, or farther side. Thus, the sound is more intense at the closer ear than the farther ear. This IID changes with the location of a sound signal.

The second mechanism is the directional and frequency dependent features of pinna. The complex folds and structures of the pinna act to enhance certain frequencies and attenuate some others in a directionally dependent manner (Roffler and Butler 1968; Searle, Braida et al. 1975). Thus if two frequencies, frequency 1 and 2, were broadcast at the same intensity from a location on one side, frequency 1 might actually be more intense at the tympanic membrane than the intensity at which it was broadcast, while frequency 2 might be less intense. The same two frequencies broadcast from a different location would generate a different set of intensities at the tympanic membrane. Furthermore, the degree of amplification or attenuation of a given frequency would be different at each ear. Thus, two frequencies that have the same intensity and come from the same location will generate different IIDs.

An additional complication is that the pinna cannot generate a unique IID for each frequency for every location. Thus, a given frequency will generate the same IID at more than one location. Therefore, the IID of a single frequency cannot accurately define the sound location since several different locations may generate the same IID. This problem is overcome by evaluating the IIDs of several frequencies. If we consider a more complex sound source composed of three frequencies, each one having the same intensity, each frequency will generate a different IID even though they all emanated

from the same location, thus generating a combination of three IIDs. If the sound moves to a different location, a new combination of IIDs will be generated by the three frequencies. Thus any location, in both azimuth and elevation, will generate a unique combination of IIDs, and it is the IIDs of at least three frequencies that provide the cue for accurately localizing a sound source (Grinnell and Grinnell 1965; Fuzessery and Pollak 1984).

An exception to this rule is for determining the elevation of sounds along the midline, directly ahead. These sounds are equally intense at two ears and generate an IID of 0 dB. Since IIDs do not change with elevation along the midline, sound elevation is not localized by IIDs but rather by the relative intensities of the frequencies in a broadband signal. The pinna can amplify certain frequencies and attenuate others in a directionally (elevation) dependent manner. So a broadband sound source located at different elevations on the midline will generate a systematic shift of the notches in the spectrum. The spectral location of the notches are created by the pinna and contain the information required for determining the elevation of a broadband sound along the midline and for distinguishing sounds located in the front from those that are located behind the head. Indeed, since IIDs do not change with midline elevation, listeners can accurately localize the elevation of sounds along the midline with only one ear.

IIDs ARE FIRST CODED IN LSO

Sound intensities received at the ears are first coded by the firing rate of the auditory nerves. The spike trains are then transmitted to the cochlear nucleus. The bushy cells of the cochlear nucleus are especially important because they then send their projections to the lateral superior olive (LSO) which is the first place where the coded intensities from two ears are compared. The comparison is a subtractive process (Caird and Klinke 1983; Moore and Caspary 1983; Joris and Yin 1995; Park, Grothe et al. 1996; Park, Monsivais et al. 1997; Casseday 2002). Signals from one ear excite the LSO and signals from the other ear inhibit it. The LSO receives excitatory projections from the ipsilateral cochlea nucleus while receive inhibitory innervation from the contralateral cochlea nucleus. This inhibition is indirect because the contralateral cochlea nucleus projects to the medial nucleus of the trapezoid body (MNTB), and MNTB then provides the LSO with glycinergic, inhibitory innervation (Moore and Caspary 1983; Glendenning, Hutson et al. 1985; Cant and Casseday 1986). In this way, each LSO cell receives excitation from one ear and inhibition from the other ear. These cells are so called excitatory/inhibitory (EI) neurons and they are sensitive to intensity disparities (IIDs). The EI cells express the comparison of the sound intensities received at two ears in their firing rates (Casseday, Kobler et al. 1989) (Fig 1.2).

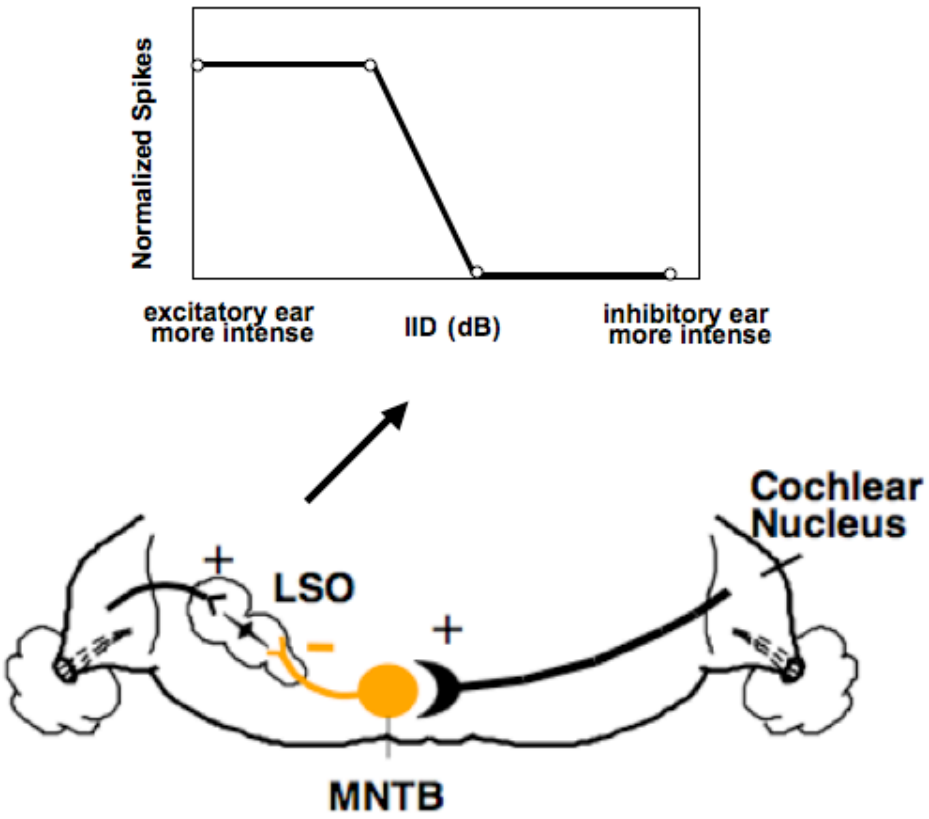

Fig 1.2. Formation of EI property in the LSO. LSO receive direct excitatory projections from cochlea nucleus (CN) on same side, and receives inhibitory projections from the contralateral CN via MNTB. Thus, LSO neurons subtractively process IIDs. The graph on the top shows a schematic IID function of a LSO cell. Sound at the ipsilateral ear drive the neuron resulting a high spike count. As the intensity is raised at the inhibitory ear, discharges are suppressed. LSO=Lateral Superior Olive.

Because the auditory system is tonotopically organized, the excitatory and inhibitory inputs that LSO neurons receive are driven by the corresponding regions of basilar membrane on each side. Thus, the coded intensity produced by a given frequency received at one ear is subtracted by the inhibition evoked by the intensity of same frequency received at the other ear.

The coding of interaural intensity disparities (IIDs) is studied by driving LSO cells with a sound of a fixed intensity at the excitatory ear and presenting sounds of increasing intensities to the inhibitory ear. As sound intensity at the inhibitory ear increases, there is a progressive suppression of the discharges evoked by the sound at the excitatory ear. One key feature that distinguishes each LSO cell is the particular IID at which the discharge is completely suppressed. Each LSO neuron is sensitive for a particular IID, the intensity difference that generates maximal suppression. We call the function that plots the spike count evoked at different IIDs as a cell's IID function (Fig 1.3).

The IID of maximal suppression is a key feature because the population of isofrequency EI cells presumably contains the full complement of IIDs of maximal suppression. Thus a frequency that emanates from a particular location in space would generate an IID that would suppress some neurons but not the others. Assuming the EI neurons within an isofrequency contour are topographically arranged according to their IIDs of maximal suppression, then a particular IID generated by a frequency would create

a border separating discharging neurons from suppressed neurons. The location of the border would shift with IID, and thus the location of the border would code for the IID generated by the particular frequency. Due to the directional properties of the pinna together with the shadowing produced by the head and ears, a broadband signal that emanates from a particular location in space would generate different IIDs for the various frequencies that comprise the signal. The IID of each frequency would be encoded by the border separating the active from the inactive cells in each isofrequency contour. In this way, the location in both azimuth and elevation could be encoded by the LSO population.

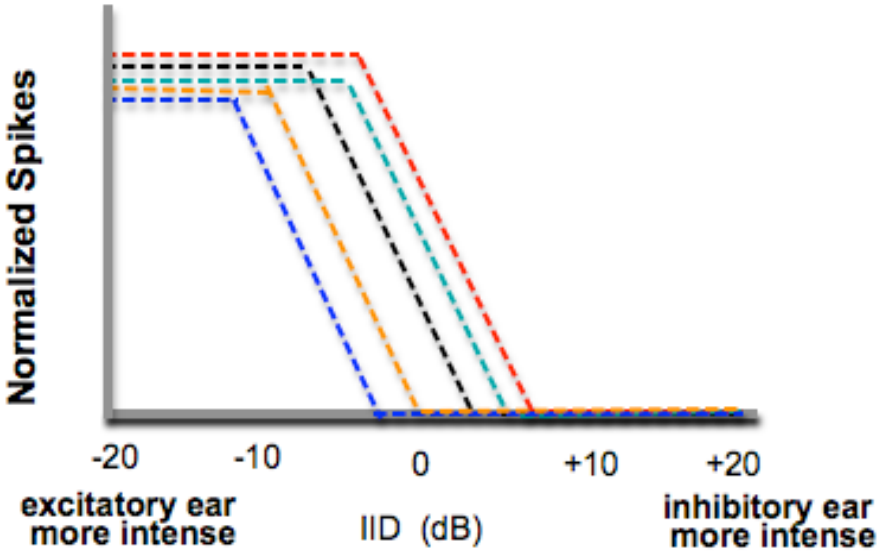

Fig 1.3. IID functions of a population of LSO cells. IID function is different for each LSO cell, depending on the threshold of inhibitory inputs innervating the LSO cell. In some LSO cells, a sound has to be louder at the inhibitory ear to completely suppress spikes. In some other cells, a sound which is louder at the excitatory ear can completely suppress spikes.

IID PATHWAY

Once the computation is made in the LSO, the coded information is conveyed to the nuclei above it. The LSO sends projections bilaterally to both the inferior colliculus (IC) and to the dorsal nucleus of the lateral lemniscus (DNLL). The bilateral projections from LSO to both the DNLL and IC use different neurotransmitters (Glendenning, Baker et al. 1992). The crossed projections, from LSO to both the contralateral DNLL and IC are glutamatergic and thus excitatory. The ipsilateral projection to both DNLL and IC, however, are mostly glycinergic and thus inhibitory. Therefore the DNLL receives excitation from the contralateral LSO and inhibition from the ipsilateral LSO. The DNLLs are also reciprocally connected to each other through the commisure of Probst (Shneiderman, Oliver et al. 1988; Shneiderman, Stanforth et al. 1999). Similarly, the IC also receives excitation from the contralateral LSO and glycinergic inhibition from the ipsilateral LSO (Fig 1.4).

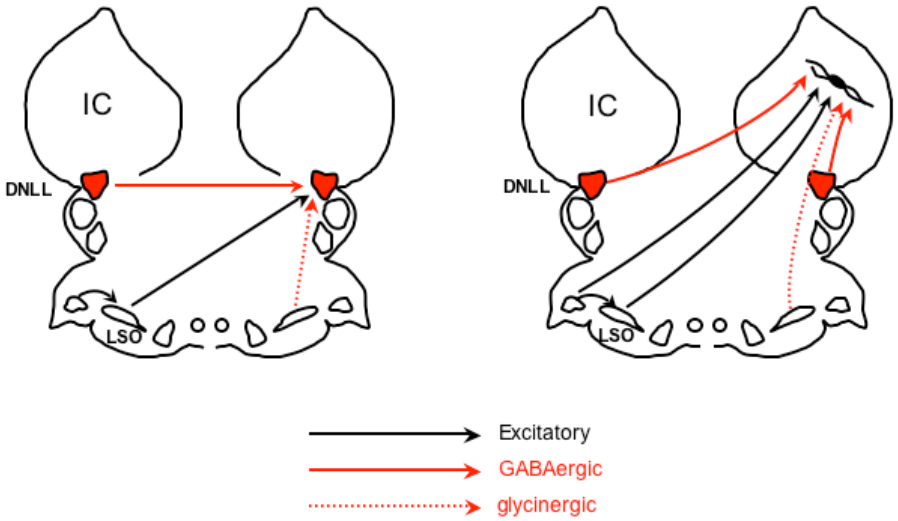

Fig 1.4. The IID pathway. The graph on the left shows the major inputs to the DNLL. The DNLL receives primary inputs from the contralateral LSO. The ipsilateral LSO projects to the DNLL with glycinergic projections. The DNLL also receives GABAergic inputs from the contralateral DNLL. The graph on the right shows the major inputs to the IC. IC receives bilateral inputs from the LSOs. Crossed projections are excitatory and the ipsilateral projections are inhibitory. The IC also receives a bilateral GABAergic inputs from the DNLLs. In addition to these binaural inputs, the IC also receives monaural inputs from the contralateral cochlea nucleus (CN).

The DNLL is located just below the IC and sends strong GABAergic projections to the IC just above it and to the opposite IC. The IC is of particular interest because it receives the projections not only from the two LSOs and DNLLs, but also from most of lower auditory nuclei, and thus is the nexus of the auditory system (Oliver 1992; Casseday 2002) (Fig 1.4). Consistent with its innervations from the LSOs, many IC cells express EI properties strikingly similar to those in the LSO (Roth, Aitkin et al. 1978; Wenstrup, Ross et al. 1986; Irvine and Gago 1990; Kelly, Glenn et al. 1991; Oliver 1992) (Fig 1.5)

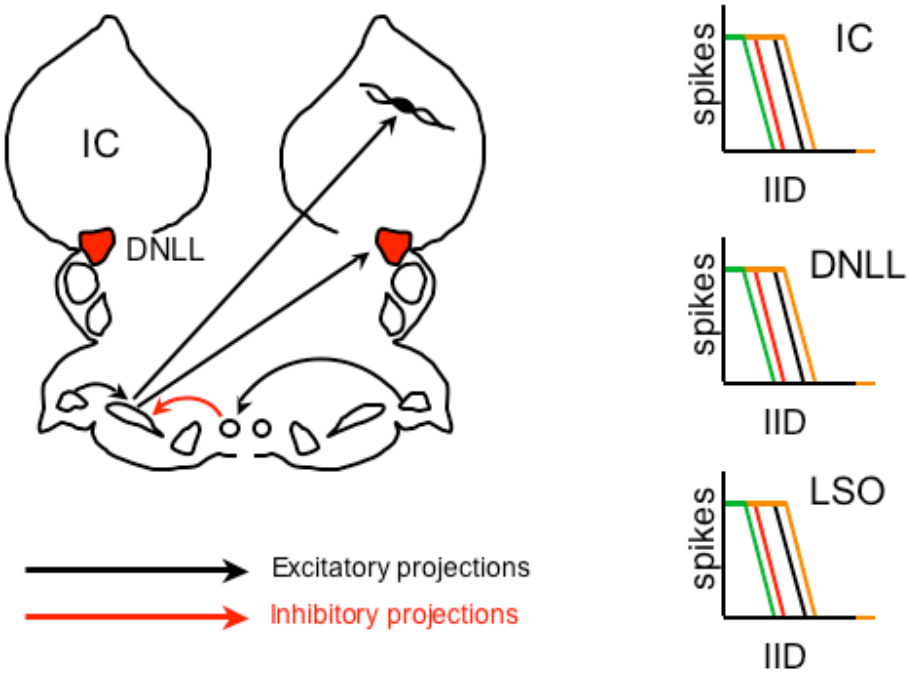

Fig 1.5. DNLL and IC have similar IID function with the LSO cells. The graph on the left shows the excitatory projections from LSO to DNLL and IC. The DNLL and IC inherited the IID function of the LSO cell from which they received innervations. The figure on right shows IC and DNLL have the similar range of IID functions with LSO cells. DNLL =dorsal nucleus of lateral lemniscus.

EI PROPERTIES IN THE IC

The IC of the mustache bat is similar in many respects to the IC of more commonly studied mammals. However, the isofrequency contour devoted to processing 60kHz is greatly expanded. Of particular significance is that monaural and binaural neurons are segregated into distinct regions. Ross and Pollak made an iontophoretic deposit of HRP in the EI region to determine the locations and numbers of retrogradely labeled cells in the auditory brainstem. They found that the inputs to the EI region originate primarily from the DNLL and LSO bilaterally and from INLL ipsilaterally. Since the LSO projects strongly to the IC and the EI properties of IC cells are so similar to those in the LSO, it follows that the EI properties of IC cells are most likely inherited from the LSO. But if the excitatory projection from LSO to IC is sufficient to account for the EI properties in the IC, why are the projections to the EI cells in the IC so complex and what is the functional impact of those projections?

Those questions prompted researchers to selectively block inhibitory inputs in the IC. The rationale was that if the EI property is formed in the LSO and is imposed on their targets in the IC, then blocking inhibition should not change the EI property, because the inhibition occurred in the LSO and not in the IC. Conversely, if the EI property is actually formed or shaped in the IC, then blocking inhibition should either transform an EI cell into a monaural cell that is uninfluenced by ipsilateral stimulation or cause a pronounce shift in the IID function.

By blocking inhibition in the IC, studies in both rats and bats found that EI cells in the IC actually comprise a diverse group, even though they exhibit common binaural response properties that are similar to LSO cells (Li and Kelly 1992; Faingold, Anderson et al. 1993; Park and Pollak 1994; Burger and Pollak 2001). The diversity is due to the variety of ways by which convergent projections innervate the population of IC cells. The projections from the DNLL are especially noteworthy since they play a prominent role in shaping or modifying the EI properties in the IC. The consequences of this large inhibitory input from DNLL to IC are substantial.

Many EI cells in the IC apparently inherit their EI property via excitatory projections from the LSO, since blocking inhibition at the IC with bicuculline and strychnine, or reversibly inactivating the DNLL, failed to change their EI property IC (Fig 1.6A).

In many other IC cells, their EI properties are created in the IC by an excitatory projection from a lower monaural nucleus and a GABAergic inhibitory projection from the opposite DNLL that is driven by sound at the inhibitory ear. This *de novo* construction was shown both by blocking inhibition at the IC and/or reversibly inactivating the opposite DNLL. These manipulations transformed EI cells that were strongly inhibited by stimulation of the inhibitory ear into monaural cells, whose responses were unaffected by stimulation at the ear that previously had been inhibitory (Fig 1.6B).

The third type is hybrid, in that blocking inhibition did not abolish the EI property but rather changed in the IID that evoked the criterion suppression. The EI property in these cells was apparently inherited from the LSO but inhibition then suppressed discharges at lower intensities at inhibitory ear and thereby adjusted the IID that evoked a complete spike suppression (Fig 1.6C).

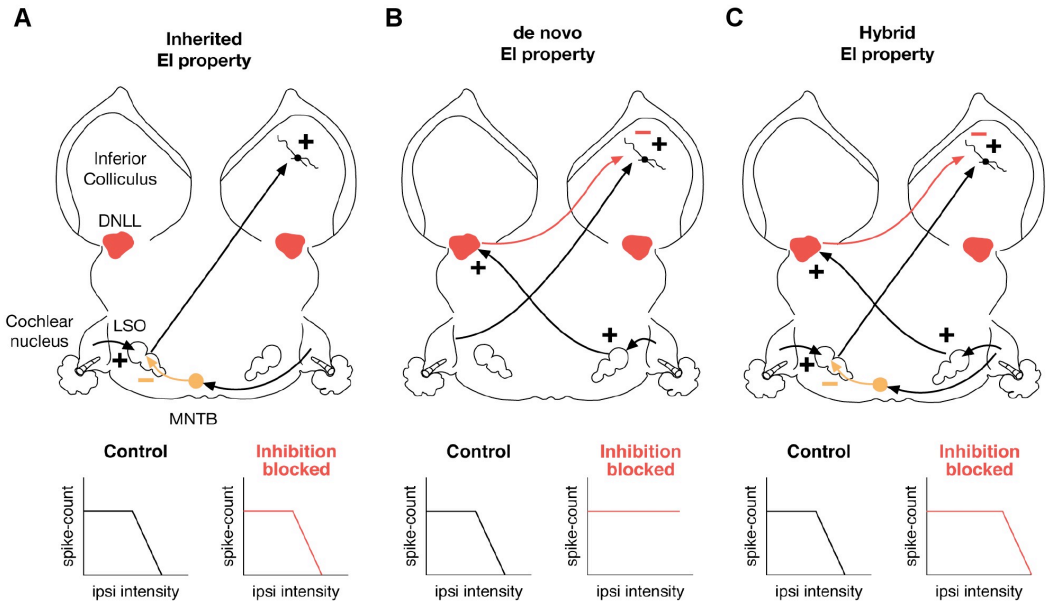

Fig 1.6. Circuitry proposed in previous reports to account for the changes in binaural properties of EI cells when inhibition was blocked or the DNLL contralateral to the IC was inactivated. Black lines show excitatory and red lines show inhibitory projections. Lower graphs show changes in IID functions obtained before and while inhibition was blocked. **A:** EI property is assumed to be inherited from the LSO when there is no change in the IID function while inhibition is blocked at the IC. **B:** EI property is created in IC from excitatory projections evoked by contralateral stimulation and inhibitory projections from the opposite DNLL that are activated by ipsilateral stimulation. In these cells blocking GABAergic inhibition at the IC or inactivating the opposite DNLL greatly reduces or completely abolishes the ipsilateral evoked spike suppression and transforms an EI into a monaural cell. **C:** Hybrid formation of EI properties. In these cells the EI property is inherited from the LSO but the inhibitory projections from the DNLL cause spike suppression at lower ipsilateral intensities than those generated by the LSO. Hence blocking inhibition or reversibly inactivating the DNLL does not abolish the EI property but cause a shift in the IIDs that generate the spike suppression.

EI/F CELLS IN THE IC

In addition to the conventional LSO type of EI cell, a new type, the facilitated EI cell (EI/f), first emerges in the IC. With EI/f cells, the spike-counts evoked by binaural signals with low intensities at the inhibitory ear are significantly lower than those evoked by the contralateral ear alone. As intensity at the inhibitory ear increases, the spike counts at first increase and peak at a particular IID, and then are suppressed as intensity at the inhibitory ear is further increased, in a manner similar to the conventional EI cells (Fig 1.7). EI/f cells are therefore selective for one IID, or a small range of IIDs, and respond maximally to sounds that emanate from highly restricted regions of space.

In a study by Park and Pollak (Park and Pollak 1993), blocking GABAergic inhibition with bicuculline eliminated the facilitation and transformed these EI/f cells into conventional EI cells. In addition to abolishing the facilitation, blocking inhibition in some EI/f cells also caused a shift in the IID that produced criterion suppression, an effect similar to that described above for conventional EI cells. They proposed a circuit that could explain the property of EI/f cells (Fig 1.8). These cells receive excitatory innervations from the opposite LSO, and also receive inhibitory projections from the ipsilateral DNLL. The assumption is that the ipsilateral DNLL cell is inhibited at a lower ipsilateral intensity, or a smaller IID than the LSO cell. They proposed that binaural stimulation with a low ipsilateral intensity (a small IID) evoked both excitation from the opposite LSO and inhibition from the ipsilateral DNLL. The inhibition from the DNLL

suppressed the excitation from the LSO, thereby producing the low spike counts in the IC cell at IIDs that favored the contralateral ear. The assumption is that the DNLL is suppressed at a lower IID than that which suppresses the LSO. Thus, when the ipsilateral intensity increases and generates a higher IID, the DNLL is inhibited but the LSO is not. Under these conditions, an increased spike count is evoked at the IC cell because the excitation from the LSO is unopposed by DNLL inhibition. At yet higher ipsilateral intensities, both DNLL and LSO cells are suppressed thereby reducing and then completely eliminating any discharges from the IC cell. Since a binaural stimulation evokes the most vigorous response only over a small range of IIDs, previous studies suggested that functionally, these EI cells selectively respond to sounds that emanate from highly restricted regions of space.

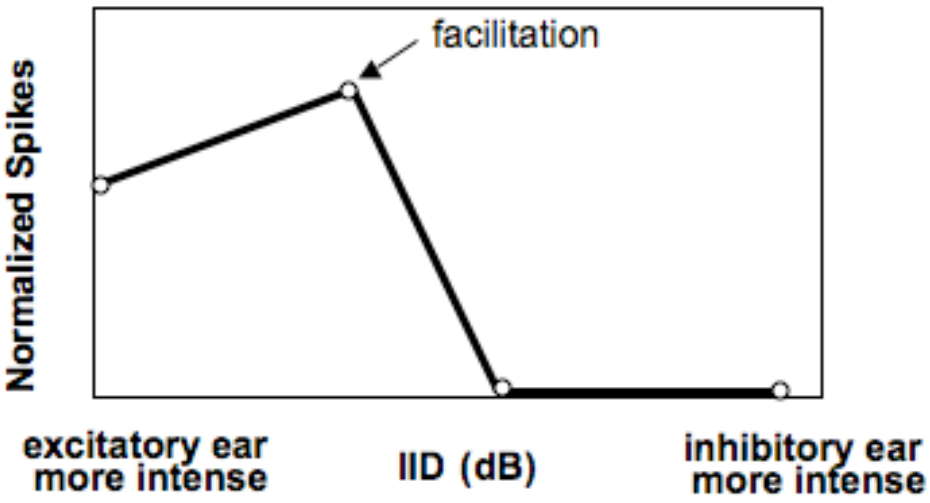

Fig 1.7. A schematic IID function of an EI/f cell. A sound at the excitatory ear drives the neuron resulting a high spike count. As the intensity is raised at the inhibitory ear, discharges first increase, causing a facilitation of the discharge. When the intensity at the inhibitory ear further increases, the discharges are suppressed.

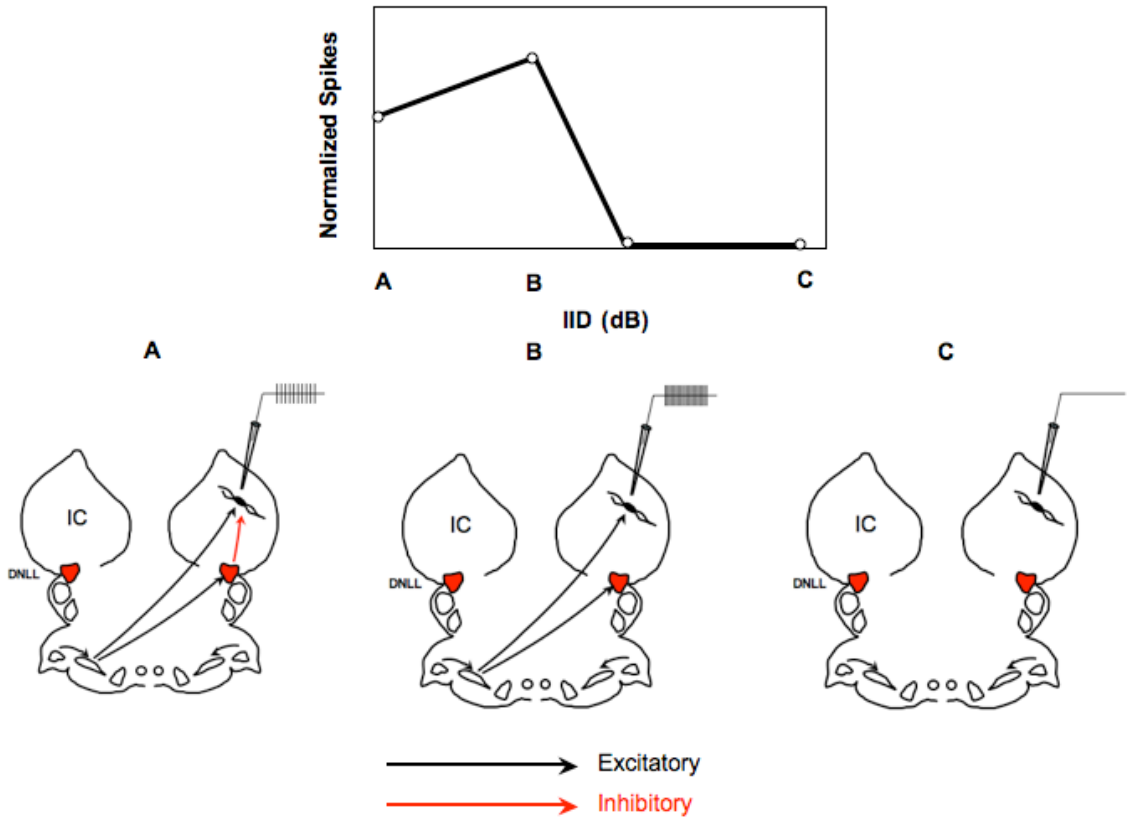

Fig 1.8. Mechanism producing EI/f cells proposed by previous studies. **A:** A binaural stimulation with a low ipsilateral intensity evoked both excitation from the opposite LSO and inhibition from the ipsilateral DNLL. The inhibition from the DNLL suppressed the excitation from the LSO, thereby producing the low spike counts in the IC. **B:** A binaural stimulation with a high ipsilateral intensity suppressed the DNLL but not the LSO. An increased spike count is evoked at the IC cell because the excitation from the LSO is unopposed by DNLL inhibition. **C:** At a higher ipsilateral intensity (a large IID), both DNLL and LSO cells are suppressed thereby completely eliminating any discharges from the IC cell.

THE FUNCTIONAL RELEVANCE OF PARALLEL PROCESSING OF IIDS

It is unclear why EI properties should be modified or formed de novo in the IC when a large population of EI cells have already been formed in the LSO. One hypothesis is that the inhibitory projections from DNLL is important in responding to multiple sound sources that emanate from different regions of space. This hypothesis is proposed by studies on mustache bats (Yang and Pollak 1994). They showed that the reception of a first signal reconfigures the circuitry of the IID pathway by functionally inactivating the DNLL for a period of time. Based on their results, during the period when DNLL is inhibited, EI cells were deprived of their inhibitory inputs from the DNLL. Therefore, the IC cells were temporarily transformed from strongly inhibited EI cells into weakly inhibited EI cells or even monaural cells. Their studies suggested that the DNLL might impart on its IC targets an ability to differentially process IIDs for multiple sound signals (Fig 1.9).

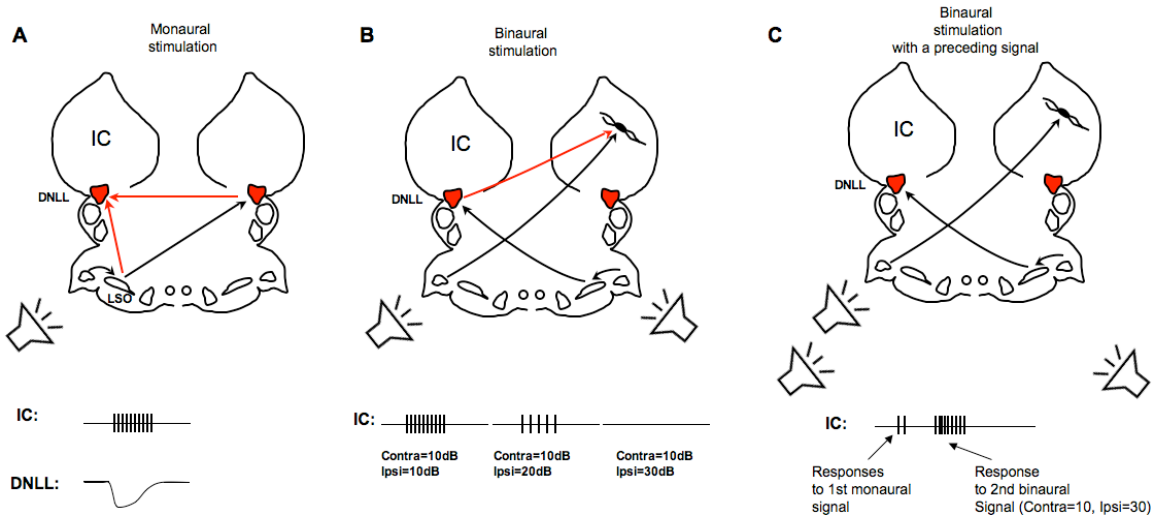

Fig 1.9. Model of acoustic inactivation of the DNLL. **A:** A persistent inhibition evoked in the DNLL by an intense monaural signal. **B:** Responses to a binaural signal decreased with increasing the intensity of the ipsilateral signal. The decrease in response was due to the interaction between the inhibitory inputs evoked by ipsilateral signal and the excitation evoked by contralateral signal. All responses were suppressed with the binaural signal with the ipsialteral intensity at 30dB SPL (Contra=10dB SPL). **C:** If a monaural signal was presented to the contralateral ear preceding the binaural signal (Contra=10dB SPL, Ipsi=30dB SPL). The IC cell not only fired to the preceding monaural signal but also discharged to the trailing binaural signal to which it did not respond. The preceding monaural signal inactivated the DNLL, and deprived the inhibition to the IC which opposed the excitation evoked by the contralateral signal and thus preventing the cell from firing.

THE PRECEDENCE EFFECT

The precedence effect was discovered in psychological studies on humans and reflects the dominance of the directional cues of the first sound received over directional cues of following sounds for localization (Wallach, Newman et al. 1949; Zurek 1980; Blauert, Canevet et al. 1989). In most studies of the precedence effect, signals are presented from two speakers separated in space. The perceived location of the signal depends on the interval between them. The location of the sound source is attributed only to the location of the initial signals. The second signal contributes to the quality of the sound, but it does not influence the perceived location. Whether the listener hears a single composite sound or two separate sounds depends on the delay between the two sounds, as well as the duration and the complexity of the sound. If the interval between the first and second sounds exceeds an upper limit, the two sounds are on longer heard as a single sound but as two separate sounds in succession, each with a perceived location in space. The hypothesis proposed by Yang and Pollak relates to a precedence effect because an initial signal change the population coding of IIDs to a trailing signals in the IC, and thus the code for the trailing signals is degraded.

The dominance of the directional cues of the first sound received over directional cues of following sounds for localization has been demonstrated in insect (Wyttenbach and Hoy 1993), birds(Keller and Takahashi 1996), and in a variety of mammals (Cranford and Oberholtzer 1976; Wickesberg and Oertel 1990; Litovsky and Yin 1998;

Litovsky and Yin 1998). Thus, it apparently is a widespread feature of auditory system. This suggests that the value of precedence for sound perception by humans in reverberant environment is a manifestation of a more general process that could enhance an animal's ability to focus on one sound in the midst of many sounds. Focus is achieved by localizing only the first sound and combine the first and trailing sounds into a single perception.

PURPOSE OF MY STUDIES

Previous extracellular studies proposed specific excitatory and/or inhibitory events that should be evoked by each ear and thereby generate each of the EI or EI/f discharge properties. The proposals were inferences based on the well established response features of neurons in lower nuclei, the projections of those nuclei, their excitatory or inhibitory neurochemistry, and the changes in response features that occurred when inhibition was blocked.

In this study we will record the inputs, the postsynaptic potentials, discharges evoked by both monaural and binaural signals in EI and EI/f cells with *in vivo* whole cell recordings from the IC in awake bats. Furthermore, We will compute the excitatory and inhibitory synaptic conductances from the recorded PSPs. Our goal is to test the synaptic events proposed by previous extracellular studies, and evaluate to what degree they are consistent with what were proposed before.

CHAPTER 2: *INTRACELLULAR RECORDINGS REVEAL NOVEL FEATURES OF EI CELLS*

INTRODUCTION

Interaural intensity differences (IIDs) are the cues that animals use to localize high-frequency sounds (Erulkar 1972; Erulkar 1972; Mills 1972). The intensities received at the two ears are coded in the auditory nerve and are first "compared" by binaural neurons in the lateral superior olive (LSO). The comparison is subtractive, whereby signals from one ear excite and signals from the other ear inhibit the binaural cells, and thus these excitatory/inhibitory (EI) neurons are sensitive to intensity disparities (Caird and Klinke 1983; Caird and Klinke 1983; Moore and Caspary 1983; Joris and Yin 1995; Park, Grothe et al. 1996; Park, Monsivais et al. 1997; Casseday 2002).

The LSO sends its axonal projections bilaterally to both the inferior colliculus (IC) and to dorsal nucleus of the lateral lemniscus (DNLL). The DNLL is located just below the IC and sends strong GABAergic projections to the IC just above it and to the opposite IC. The IC is of particular interest because it receives the projections not only from the two LSOs and DNLLs, but also from most other lower auditory nuclei, and thus

is the nexus of the auditory system (Oliver 1992; Casseday 2002). Consistent with its innervation from the LSO, many IC cells express EI properties similar to those in the LSO (Roth, Aitkin et al. 1978; Wenstrup, Ross et al. 1986; Irvine and Gago 1990; Kelly, Glenn et al. 1991; Oliver 1992).

The EI cells in the IC, however, comprise a diverse group, even though they exhibit binaural response properties similar to LSO cells. The diversity is apparent from the changes in EI properties when inhibition at the IC is blocked or the DNLL is reversibly inactivated (Li and Kelly 1992; Faingold, Anderson et al. 1993; Park and Pollak 1994; Burger and Pollak 2001). Previous studies proposed specific excitatory and/or inhibitory events that should be evoked by each ear and thereby generate each of the various types of EI cells in the IC via the projections shown in Fig 1.6. The synaptic events proposed for each of the circuits, however, could not be directly observed with extracellular recordings but rather were inferred from the changes in discharge properties due to blocking inhibition. To evaluate the degree to which the proposed synaptic events actually occur, we used in vivo whole cell recordings from the IC in awake bats to directly visualize both the inputs to EI cells, as revealed by sound evoked excitatory and inhibitory postsynaptic potentials, and the outputs, the discharges evoked by both monaural and binaural signals. We show that in a minority of cells the PSPs evoked by monaural and binaural signals are consistent with the synaptic events proposed in previous extracellular studies to account for the various formations of EI properties. In the majority of EI cells, however, ipsilateral signals evoked subthreshold EPSPs. The

EPSPs behaved paradoxically in that the EPSP amplitudes increased with intensity, even though binaural signals with the same ipsilateral intensities generated progressively greater spike suppressions. These additional subthreshold responses not only show that the circuitry underlying EI cells is more complex than previously suspected but also suggest that the additional EPSPs could influence the responsiveness of EI cells to signals that generate IIDs that change over time, such as moving sound sources or multiple sounds that occur in complex acoustic environments.

METHODS

Surgical Procedures

Experiments were conducted on male Mexican free-tailed bats, *Tadarida brasilensis mexicana*, captured from local sources in Austin, Texas. Surgical procedures were as described in previous reports (Xie, Gittelman et al. 2008; Gittelman 2009). In brief, bats were sedated with Isoflurane (inhalation) and then anesthetized with an intraperitoneal injection of ketamine/xylazine (75 - 100 mg/kg Ketamine, 11 - 15 mg/kg Xylazine, Henry Schein, Inc. Melville, NY). The muscles and skin overlying the skull were reflected, a foundation layer of cyanoacrylate was placed on the surface of the skull and a small metal rod was cemented to the foundation layer on the skull and then attached

to a bar. The IC in bats is hypertrophied and can be seen through the thin brain case protruding between the cortex and cerebellum. An opening was made in the skull over the IC and the brain was kept clean and moist with Ringer solution. The bat was placed in a restraining cushion, placed in the stereotaxic device, and the bar on its head was secured to the stereotaxic. Recordings were begun after the bats recovered from the anesthetic and thus all data were obtained from awake animals. The bats typically lay quietly during the remainder of the experiments. If they showed signs of discomfort, data collection was stopped and doses of the neuroleptic, ketamine hydrochloride (1/40 dilution, 0.01cc injection, Henry Schien, Inc., Melville, NY), were administered. If they continued to show signs of discomfort, the experiments were terminated. All experimental procedures were in accordance with a protocol approved by the University of Texas Institutional Animal Care Committee.

Acoustic Stimuli

Auditory stimuli were tone bursts generated digitally in IGOR-PRO. Tone bursts had durations of 5-20 ms and rise-fall times of 0.2 ms. The acoustic signals were sent to an Instrutech 16-bit D/A converter and were fed to custom made electronic attenuators and then to custom designed earphones. The frequency characteristics of each earphone was determined with a ¼ inch Bruel and Kajer microphone and the computer compensated for output fluctuations across frequency so that each earphone was flat $\pm$ 2 dB from about 10-50 kHz. At the start of each experiment, the earphone was inserted

into the funnel formed by the bat's pinnae and positioned adjacent to the external auditory meatus. The acoustic crosstalk with this arrangement is about 40 dB.

Recording Procedures and Data Acquisition

Responses were recorded with patch electrodes (5-10 MΩ) pulled from thick walled (1.65 mm OD, 1.1 mm ID) capillary glass (WPI, #PG52165-4, Sarasota, Florida). The standard internal solution was (in mM): K-gluconate (115), HEPES (10), KCl (7), MgATP (4), Na_2GTP (0.3), EGTA (0.5), Na_2Phosphocreatine (10). Membrane potentials were not corrected for liquid junction potentials.

During experiments, the electrodes were positioned over the IC and lowered into the IC with a piezoelectric microdrive (Burleigh Inchworm; EXFO Burleigh, Plano, TX) while under positive pressure of 2 - 3 PSI. Electrodes were lowered to a depth of ~ 300 μ to bypass the external nucleus of the IC and ensure recordings were made from cells in the central nucleus of the IC. All cells were recorded at depths of 300-1000μ from the surface of the IC. Upon entering the central nucleus, the pressure was reduced to 0.3 - 0.7 PSI and the electrodes were advanced in steps of 1-2 μ. Cell search was conducted in voltage clamp mode using a -5 mV step to monitor electrode resistance. When contact with a cell was made, pressure was released and a small amount of negative pressure (< 0.5 PSI) was applied to obtain a giga-ohm seal. After a seal was obtained, additional negative pressure was applied to break-in, and the amplifier was switched to whole-cell current clamp mode, the voltage offset was set to 0 and the electrode capacitance

neutralized. Recordings evoked by sound were then obtained. Responses were sent either to a Dagan BVC 700A Bridge and Voltage Clamp Amplifier (Minneapolis, MN) in earlier experiments, or to a MultiClamp 700 B Microelectorde Amplifier (Axon Instruments/Molecular Devices, Union City, CA) and then to an InstruTech ITC-18/PCI (Port Washington, NY) A/D/A converter (200 kHz sampling rate), and stored on a Macintosh G5 computer (Cupertino, CA). Analyses were done in IGOR PRO. Tone bursts were first presented to the contralateral ear and frequency was manually scanned to determine the cell's best frequency (BF), the frequency at which the lowest intensity evoked discharges. BF tones were then presented to evaluate responses evoked by contralateral, ipsilateral and binaural stimulation. Each tone was presented 8-20 times. In each of the figures below, the records that show EPSPs with spikes are from a single tone presentation while each subthreshold PSP shown is the average of the 8-20 tone presentations. The spike-counts evoked by the particular number of tone presentations are shown on the right of each suprathreshold record.

RESULTS

We recorded spikes and post-synaptic potentials (PSPs) intracellularly with patch electrodes from 42 cells in the central nucleus of the inferior colliculus (IC) of awake bats. Resting membrane potentials were not corrected for liquid junction potentials and

ranged from -45 to -57 mV and were more or less evenly distributed among the various types of EI cells described below. All responses were evoked by tone bursts having durations of 5-20 ms presented at the neuron's BF. Tones were presented monaurally over a range of intensities to either the contralateral or ipsilateral ear alone. Binaural signals were presented with the tone at the contralateral ear fixed at one intensity, usually about 10 dB above spike threshold, while tones were presented simultaneously to the ipsilateral ear over a 20-40 dB intensity range, from about 10 dB below to 30 dB above the intensity at the contralateral ear.

We recorded three major aural types of IC cells based on their responses to monaural and binaural stimulation. The three types were described in previous extracellular studies of the IC in bats and other mammals (Roth, Aitkin et al. 1978; Fuzessery, Wenstrup et al. 1985; Semple and Kitzes 1987; Li and Kelly 1992; Park and Pollak 1993). The first type is monaural. These cells, which comprised ~7% of our sample (3/42 cells), were driven by contralateral stimulation but no discharges were evoked by ipsilateral stimulation (Fig 2.1). Most importantly, the spike-counts evoked by contralateral tones were not affected by tones presented simultaneously to the ipsilateral ear, even when the ipsilateral tones were 10-30 dB more intense than the contralateral tones. The second type is excitatory-inhibitory (EI) and was the most common type we recorded (67%, 28/42 cells)(Fig 2.2). Similar to the monaural cells described above, these cells were driven only by contralateral stimulation and ipsilateral tones never evoked discharges. Unlike monaural cells, contralaterally evoked spike-counts were

inhibited when ipsilateral tones were presented simultaneously, hence the term excitatory-inhibitory. The third type is the facilitated EI cell (EI/f), which comprised 26% (11/42) of our sample (Fig 2.8). These cells differed from conventional EI cells in that binaural signals with low ipsilateral intensities evoked spike-counts at least 20% higher than the counts evoked by the contralateral signals alone. As ipsilateral intensity increased, however, spikes were progressively suppressed and were completely suppressed with ipsilateral signals 10-30 dB more intense than the contralateral signals.

In the sections below we first describe the various projection patterns that were proposed in previous studies to account for the different formations of EI cells in the IC. The projections are shown in Fig 1.6. In the subsequent sections, we focus on PSPs, both EPSPs and IPSPs. Particular attention is directed at the PSPs evoked by monaural stimulation of the ipsilateral ear. In each case we suggest how the interactions of the synaptic events evoked by each ear influenced the discharges evoked with binaural stimulation, and how those features relate to the projection patterns in Fig 1.6.

Circuits proposed in previous studies that account for the spike suppression by ipsilateral tones

All cells whose contralaterally evoked discharges were suppressed with binaural stimulation were categorized as EI or EI/f. There are, however, three explanations that can account for the suppression of contralaterally evoked discharges with binaural

stimulation, the EI property, based on the excitatory and inhibitory projections that innervate the IC (Fig 1.6) (Li and Kelly 1992; Faingold, Anderson et al. 1993; Burger and Pollak 2001; Pollak, Burger et al. 2003). The facilitation evoked by binaural signals in EI/f cells, and the projection that could explain just the facilitation is considered in the following sections. One explanation is that the EI property was formed in a lower nucleus, presumably the LSO, and that property was then imposed on the IC cell via an excitatory projection (Fig 1.6A). The EI property would, in that case, be inherited. Alternatively, the EI property may have been formed in the IC through a monaural excitatory projection evoked by sound at the contralateral ear that was then shaped by inhibitory inputs to the IC cell evoked by the ipsilateral ear, e.g., from the DNLL on the opposite side. The binaural property in this case would be created *de novo* in the IC by the interactions of the synaptic events at the IC that were evoked by stimulating each ear (Fig 1.6B). A third explanation is that yet other EI cells were hybrids, in that they had features of both types described above. In these cells the EI property is first formed in the LSO. The EI property, however, is modified in the IC through the convergence of LSO and inhibitory projections that presumably come from the DNLL. The net effect of this convergence is to create EI cells in the IC that are suppressed by lower intensities at the ipsilateral ear than they would be if they received only the LSO projection (Fig 1.6C). Below we show the PSPs that were evoked by stimulation of each ear and how their interactions could account for the binaural discharge properties observed in each cell.

We also point out the degree to which the circuits in Fig 1.6 can account for the results we observed in the various types of EI cells with patch electrodes.

Post-synaptic potentials evoked by monaural and binaural stimulation in monaural cells

In Fig 2.1 we first show the responses, PSPs and spikes, evoked in a monaural cell to provide a baseline for comparison with the EI and EI/f cells considered below. Consistent with the criteria given above for monaural cells, the spike-counts evoked by contralateral tones were not affected by tones presented simultaneously to the ipsilateral ear, and ipsilateral stimuli presented alone evoked no detectable IPSP or EPSP at any intensity. As a further check to ensure that ipsilateral stimulation did not evoke either a shunting inhibition, where the resting potential was at or near the chloride equilibrium potential, or an inhibition that canceled an excitation, we hyperpolarized the membrane potential from its normal resting potential of –55 to -73 mV (Fig 2.1B). Ipsilateral signals did not evoke a PSP during hyperpolarization at any intensity, thereby confirming that no subthreshold response was evoked by ipsilateral stimulation. Although the hyperpolarization prevented spiking, contralateral stimulation alone evoked a large EPSP due to the increase in the excitatory driving force. With binaural signals, increasing the ipsilateral intensity did not reduce the amplitudes of the EPSPs, which were equal to the amplitudes evoked by the contralateral signal presented alone (Fig 2.1C). All of these

features show that ipsilateral stimulation had no effect on the synaptic events evoked by contralateral stimulation. The finding that ipsilateral signals evoked no response, even when the ipsilateral signals were 30 dB louder than the contralateral signals, also showed that there was no speaker crosstalk at those intensities, i.e., that sound presented to one ear did not leak over to stimulate the other ear.

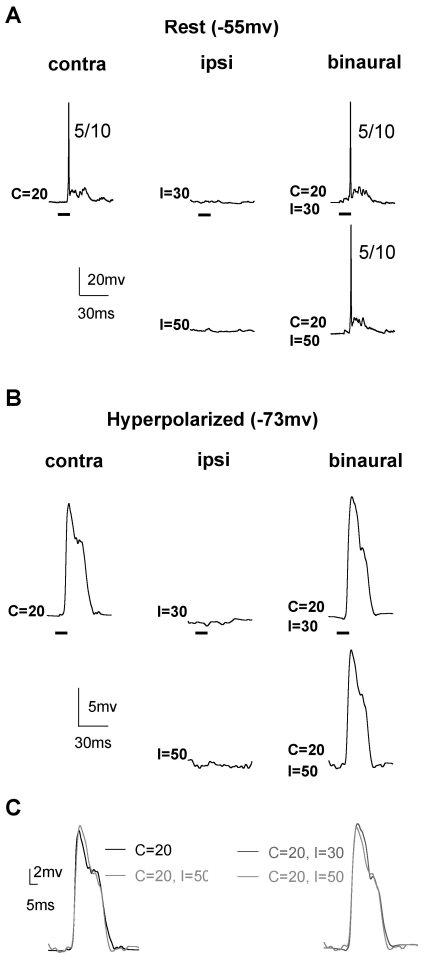

Fig. 2.1. Responses of a monaural neuron. **A:** Ipsilateral tones at 30 and 50 dB SPL evoked no response. Binaural signals that had the same ipsilateral intensities did not change the spike-count evoked by the contralateral signal. Responses were evoked at the resting potential of -55 mV. **B:** Same stimuli as in A were presented but the membrane potential was hyperpolarized from -55 to -73 mV. The hyperpolarization prevented the cell from discharging and ipsilateral tones at 30 and 50 dB SPL did not evoke responses during hyperpolarization. **C:** Overlaid PSPs in bottom records show that ipsilateral tones had no effect on contralaterally evoked PSPs. Recordings on bottom right show overlaid EPSPs evoked by binaural tones with the two IIDs. Records on bottom left show the contralaterally evoked PSP had the same magnitude and shape as the PSP evoked by the binaural signal in which the ipsilateral intensity was 50 dB SPL. Tone duration, 10 ms.

Post-synaptic potentials evoked in EI Cells

Although all EI cells were homogenous in terms of their spike suppression with binaural stimulation, three different types of EI cells were observed based on the PSPs, or absence of PSPs, evoked by ipsilateral stimulation. The first type of EI cell (5/28) was similar to monaural cells, in that no PSP was evoked by ipsilateral stimuli, although the same ipsilateral signals, when presented binaurally, suppressed the discharges evoked by contralateral signals. These features are consistent with the circuit in Fig 1.6A and are illustrated by the cell in Fig 2.2A. To ensure that the ipsilateral signals did not evoke a shunting inhibition, the cell was hyperpolarized from rest (-57 mV) to -82 mV (Fig 2.2C). Like the monaural cell described above, there were no PSPs evoked by ipsilateral stimulation while the cell was hyperpolarized. However, as the ipsilateral intensity increased during hyperpolarization, the PSPs evoked by binaural tones were progressively reduced in amplitude, and there was no discernable PSP at the highest ipsilateral intensity, i.e., when the ipsilateral tone was 20 dB SPL. Our explanation for these results is that IC cell only received excitatory inputs from a lower nucleus that was driven by stimulation of the contralateral ear. The lower nucleus, however, was binaural and had EI properties, e.g., LSO, and those properties were then imposed on the IC cell through an excitatory projection, as shown in Fig 1.6A. Thus, the ipsilateral inhibition of contralaterally evoked discharges occurred in the lower nucleus and the EI properties of these IC cells were inherited.

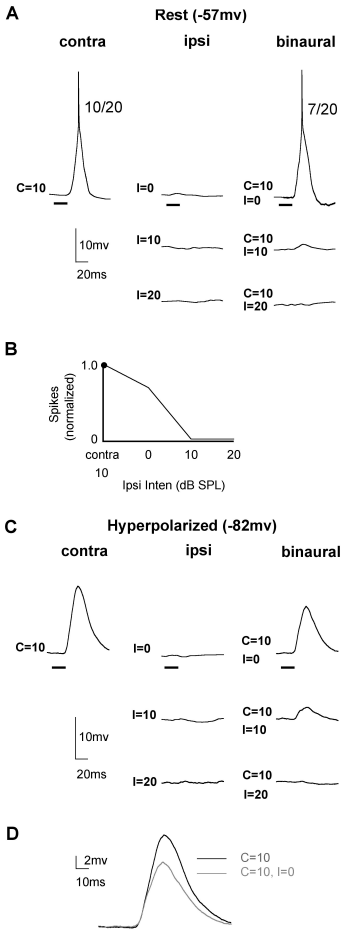

Fig 2.2. An EI cell in which the contralaterally evoked spikes were completely suppressed by ipsilateral tones, but ipsilateral tones evoked no responses. **A:** Responses evoked by contralateral, ipsilateral and binaural tones at the resting potential. Ipsilateral tones presented monaurally evoked no responses even though binaural tones completely suppressed spikes. **B:** IID function. **C:** Responses to the same stimuli while the cell was hyperpolarized to -82mV. The hyperpolarization prevented spiking and a large EPSP was evoked by the contralateral tone. Ipsilateral signals presented monaurally did not evoke any discernable response, showing that the cell did not receive ipsilateral innervation. With binaural signals, however, the EPSPs evoked by the contralateral tones were progressively suppressed by ipsilateral tones until the EPSP was abolished when the ipsilateral intensity was 20 dB SPL. **D:** EPSPs evoked by contralateral tone alone is overlaid with binaural tone showing that an ipsilateral tone of 0 dB SPL reduced the amplitude of the EPSP evoked by the contralateral tone. Tone duration, 20 ms.

The second type (3/28 EI cells) had ipsilaterally evoked IPSPs that most likely inhibited the contralaterally evoked excitation, and thereby formed the EI property in the IC, features consistent with the circuit in Fig 1.6B. Three features of the IPSPs are noteworthy and are illustrated by the EI cell in Fig 2.3. The first feature is that the ipsilateral inhibition was intensity dependent, where the IPSP magnitudes progressively increased with ipsilateral intensity in a manner that mirrored the intensity dependent spike suppression. The second is that the latencies of the IPSPs were similar to the latencies of the contralaterally evoked excitation, where the inhibition and excitation overlapped extensively and were coincident at ipsilateral intensities that caused complete spike suppression. The third feature is that the magnitudes of the EPSPs evoked by the binaural tones declined in concert with the increasing magnitudes of the IPSPs evoked when tones were presented only to the ipsilateral ear. These features are consistent with the circuitry shown in Fig 1.6B, and thus with the proposition that the EI property in these cells was formed *de novo* in the IC by the ipsilaterally evoked inhibition that suppressed the contralaterally evoked discharges.

The third type of EI cell was the most common type (20/28 EI cells) and displayed the most surprising feature. The surprising feature is that ipsilateral stimulations evoked intensity dependent EPSPs. Evidence of ipsilaterally evoked EPSPs was never detected in extracellular studies and thus was not previously proposed as a projection in EI cells.

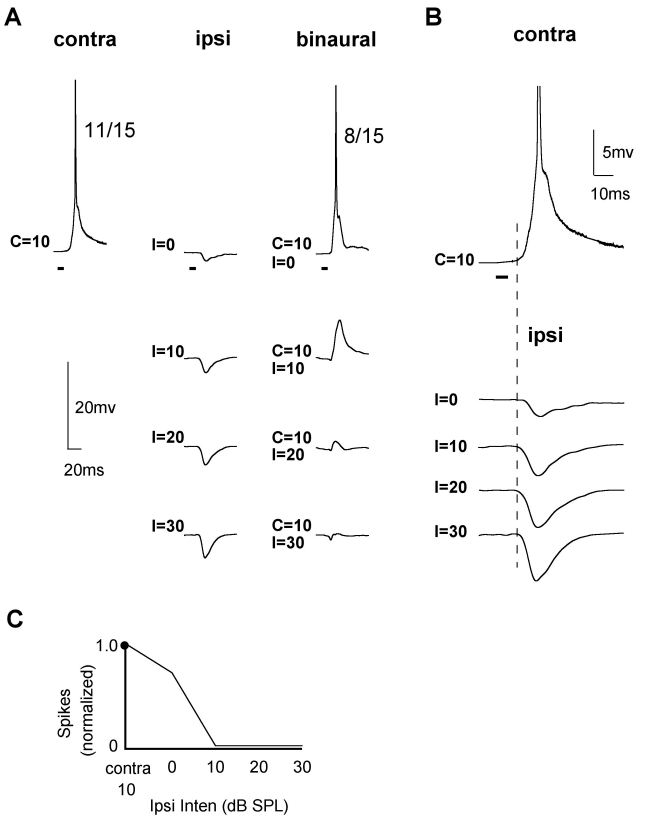

Fig 2.3. **A:** An EI cell in which a contralateral tone evoked a suprathreshold EPSP and ipsilateral signals only evoked IPSPs that increased with intensity. With binaural signals, the contralaterally evoked spikes were progressively suppressed as ipsilateral sound intensity was increased. The spike suppression mirrored the increases in IPSP amplitudes with ipsilateral intensity suggesting that the EI property was created de novo in the IC with the circuitry shown in Fig. 1.6B. **B:** Excitatory response to a 10 dB SPL contralateral tone aligned with IPSPs evoked by ipsilateral tones at different intensities showing that the latencies of the IPSPs were coincident with the EPSP evoked by a contralateral tone. **C:** IID function of spike-counts. Tone duration, 5.0 ms.

These EI cells were not homogeneous, but rather displayed one of two intensity dependent EPSP patterns. In 13/20 EI cells, ipsilateral tones only evoked EPSPs with amplitudes that increased with intensity (Fig 2.4). As explained below, the circuit that best accounts for their features is the circuit in Fig 1.6A, but with the addition of an ipsilateral excitatory projection (Fig 2.4C). The addition of the ipsilateral excitatory projection generated a paradoxical feature, the amplitudes of the ipsilaterally evoked EPSPs increased with intensity in each of the 13 cells (Figs 2.4F, 2.5), but binaural signals with the same ipsilateral intensities generated progressively greater suppressions. When presented binaurally, the highest ipsilateral intensities produced a complete spike suppression, as shown in Fig. 5A when ipsilateral intensities were 40-50 dB SPL.

Importantly, the binaural signal with the highest ipsilateral intensity, e.g., C=10, I=50 dB for the cell in Fig 2.4, evoked a subthreshold EPSP with a waveform virtually identical in latency, amplitude, shape to the EPSP evoked by a 50 dB ipsilateral signal presented alone (Fig 2.4E). What these features suggest is that the spike suppression due to binaural stimulation occurred in a lower nucleus, e.g., the LSO, and that ipsilateral stimulation activated two different projections; one projection inhibited the LSO and another projection provided subthreshold excitation to the IC (Fig 2.4C). Thus, with binaural stimulation, increasing ipsilateral intensities progressively inhibited the excitatory drive from the LSO while simultaneously increasing the subthreshold excitation from the monaural excitatory projection. With IIDs of 30 and 40 dB, ipsi ear more intense, the LSO was completely inhibited leaving only the ipsilaterally evoked

excitation. Hence, the PSP evoked by both the monaural ipsilateral signal at 50 dB and the PSP evoked by the binaural signal that had the same ipsilateral intensity (C=10, I=50 dB) were virtually the same because they were both evoked only by the ipsilateral excitatory projection.

The paradox of EPSPs evoked by both the contralateral and ipsilateral signals presented alone coupled with a complete spike suppression when the same signals were presented binaurally was also seen in 7 other cells. In those cells, however, low ipsilateral intensities evoked IPSPs that then changed into EPSPs followed by IPSPs at higher ipsilateral intensities (Fig 2.6A). The circuit that can account for the monaural and binaural properties of these cells is similar to the circuit proposed for the EI cells with only ipsilaterally evoked EPSPs, but with the further addition of an ipsilaterally evoked inhibitory projection that probably originated in the opposite DNLL (Fig 2.6C). The amplitudes of the EPSPs in these cells also increased with ipsilateral intensity and binaural signals with the same ipsilateral intensities that evoked the largest EPSPs when presented monaurally produced a complete discharge suppression. Moreover, the PSP evoked by the binaural signal with the highest ipsilateral intensity, i.e., C=30 dB SPL, I=40 dB SPL in Fig 2.6E, was similar in latency, shape and magnitude to the PSP evoked by a 40 dB ipsilateral signal presented alone, as also occurred for the neuron in Fig 2.4. As we show next, the ipsilaterally evoked EPSPs that we observed in EI cells were also seen in the EI/f cells.

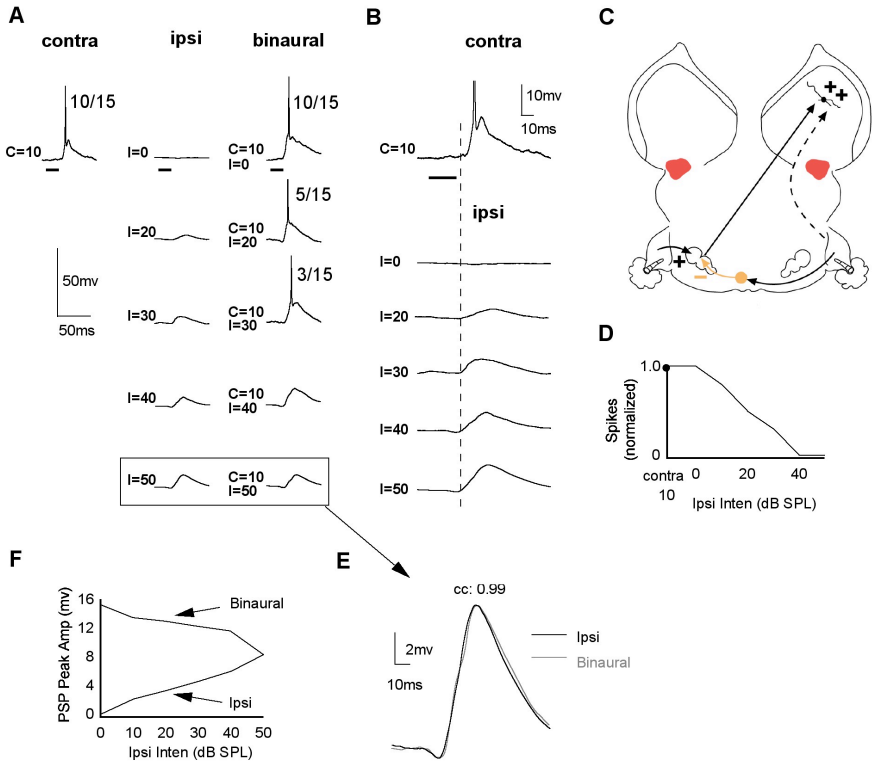

Fig 2.4. An EI cell in which ipsilateral tones evoked only EPSPs with amplitudes that increased with intensity. **A:** Responses evoked by contralateral, ipsilateral and binaural signals. Bracketed responses show the response to the ipsilateral tone at 50 dB SPL and the response to a binaural tone with the same, 50 dB SPL ipsilateral intensity. **B:** Same response to contralateral and ipsilateral tones as in panel A but contra- and ipsilateral tones are aligned in time and shown at higher magnification. **C:** Circuit that could generate the responses. **D:** IID function. **E:** The responses below the arrow are the two responses in brackets but are superimposed at a higher magnification. Amplitude of EPSPs are ~8.0 mV. **F:** Amplitudes of ipsilaterally evoked EPSPs plotted together with the amplitudes of the EPSPs evoked by binaural signals that had the same ipsilateral intensities. Note that ipsilateral EPSP amplitudes increased with sound intensity whereas the binaurally evoked EPSPs decreased with the same ipsilateral intensities. EPSP amplitudes of binaural signals were measured after spikes were eliminated. Tone duration, 20 ms.

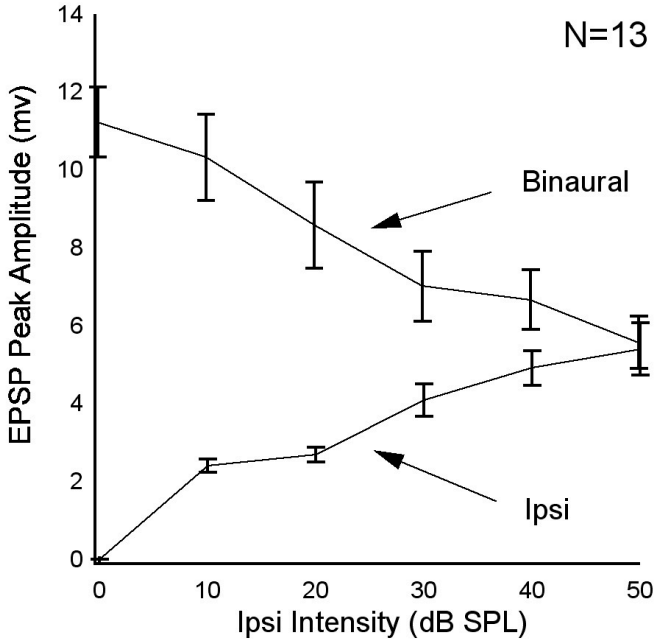

Fig 2.5. Paradoxical effects of presenting ipsilateral signals monaurally compared to the effects of presenting the same ipsilateral signals binaurally. Increases in amplitudes of the ipsilaterally evoked EPSPs with intensity were in the exact opposite direction to the reduced EPSP amplitudes evoked by the same ipsilateral intensities presented binaurally. Lines connect mean EPSP amplitudes of 13 EI cells. The EPSPs of binaural signals that evoked spikes were measured after spikes were filtered from records. Error bars show standard error of the mean.

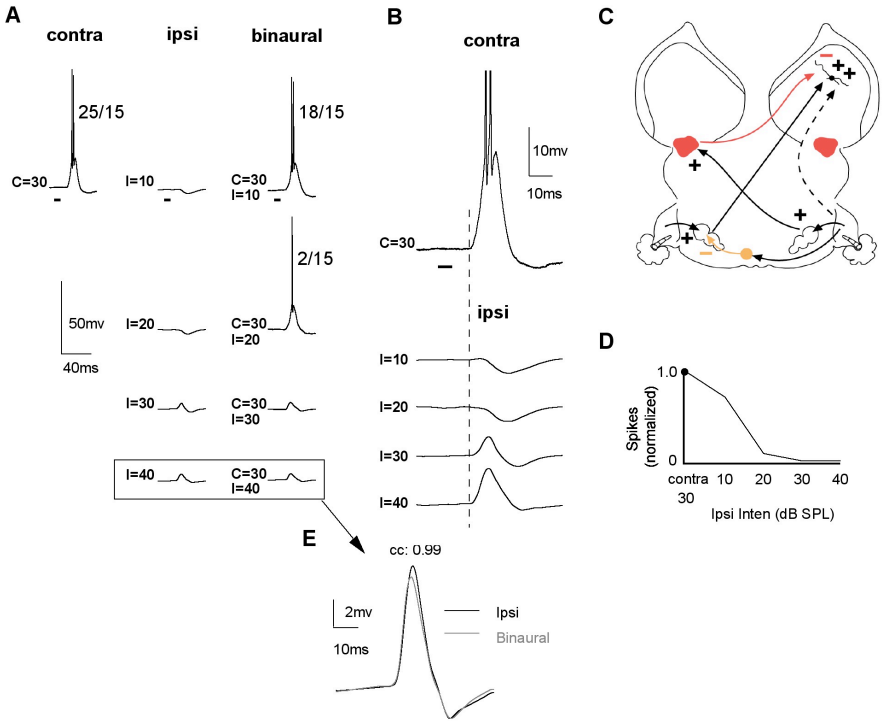

Fig 2.6. An EI cell in which increasing intensity of the ipsilateral tone first evoked IPSPs that changed into prominent EPSPs followed by a shallow IPSP at higher intensities. Panels A–E are same as in Fig 2.4. Tone duration, 5.0 ms.

Post-synaptic potentials evoked in EI/f Cells

The main difference between EI and EI/f cells is that facilitated spike-counts were evoked over a small range of IIDs at which the ipsilateral signals were either equal to or less intense than the contralateral signals. Since spike-counts with binaural tones were enhanced by at least 20% above the counts evoked by the contralateral tones alone, it would be reasonable to expect that the ipsilateral intensity that evoked facilitation should evoke an EPSP when presented alone. As expected, in most EI/F cells (7/11), ipsilateral tones evoked EPSPs when presented monaurally at the same intensity that evoked facilitation when presented binaurally (Fig 2.7A, 2.8). The EPSP amplitudes ranged from ~2-5 mV and apparently were sufficiently large that the boosts they gave to the contralaterally evoked excitations generated the facilitations. Thus the mechanism that produced the facilitation in these cells apparently was a summation of ipsi- and contralaterally evoked EPSPs.

In 4 of the 11 EI/F cells, however, low intensity ipsilateral tones presented monaurally evoked virtually no subthreshold response, even though the same ipsilateral signal generated a facilitated response when presented binaurally (Fig 2.7B). To confirm that no inputs were evoked by low intensity ipsilateral signals, we evaluated responses in two cells before and when their membrane potentials were hyperpolarized, and in both cases, low intensity ipsilateral signals evoked no responses. This is illustrated by the monaural and binaural responses of the cell in Fig 2.7B when it was hyperpolarized from

its normal resting potential of -57 mV to -70 mV. Ipsilateral tones at the intensity that evoked discharge facilitation (at 0 dB SPL) did not evoke a PSP under hyperpolarization, although binaural signals evoked a larger EPSP than was evoked by the contralateral tone alone (Fig 2.7C). It would appear that in these cells, the summation of ipsilaterally and contralaterally evoked responses could not account for the facilitation evoked by binaural stimulation. The mechanism of facilitation in these cells is unclear but the facilitation presumably was initially generated in a lower nucleus.

Binaural signals with ipsilateral intensities higher than those that produced discharge facilitation progressively suppressed spikes in all EI/f cells in a way identical to that described above for conventional EI cells (Fig 2.8), and thus the EI property, as opposed to the facilitation, is most likely generated by the same circuit proposed for conventional EI cells with ipsilaterally evoked EPSPs, i.e., Fig 1.6A. As with the majority of the conventional EI cells described previously, EPSPs were evoked by ipsilateral stimulation, and in 10 of 11 EI/f cells the amplitudes of the ipsilaterally evoked EPSPs increased with intensity. Moreover, with binaural signals the same paradox was observed in these EI/f cells as in the EI cells; the increases in amplitudes of the ipsilaterally evoked EPSPs with intensity were in the exact opposite direction to the reduced spike-counts evoked by the same ipsilateral intensities when presented binaurally (Fig 2.8A). Finally, the subthreshold EPSPs evoked by binaural signals at high ipsilateral intensities, i.e., 40 and 50 dB SPL in Fig. 2.8, had the same waveforms and amplitudes as those evoked by ipsilateral signals presented monaurally at 40 and 50 dB SPL. These

results support the proposition presented earlier, that the inputs normally activated by the contralateral ear may have been inhibited at those IIDs in a lower binaural nucleus, and that the EPSPs evoked at those IIDs were generated only by the excitatory inputs activated by the ipsilateral ear, as in the circuit shown in Fig. 2.4C.

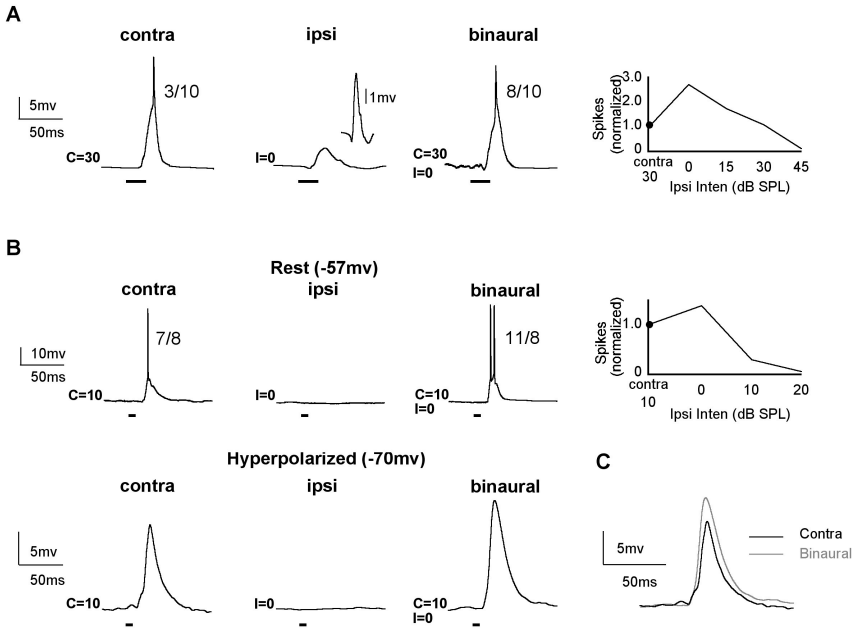

Fig 2.7. Two EI/f cells in which ipsilateral tones evoked different responses when presented monaurally. **A:** Low ipsilateral intensity (0 dB SPL) evoked an EPSP and spike-counts were facilitated with binaural signals that had the same low (0 dB SPL) ipsilateral intensity. Insert shows EPSP in higher magnification. IID function based on normalized spike-counts is shown on right. Tone duration, 20.0 ms. **B:** Another EI/f cell in which low ipsilateral intensities (0 dB SPL) did not evoke any response but spike-counts were facilitated with binaural signals that had the same ipsilateral intensity (0 dB SPL). Top panel shows responses to a contralateral tone at 10 dB SPL, an ipsilateral tone at 0 dB SPL, and the facilitated response to tones with the same intensities presented binaurally. Lower panel shows responses to the same stimuli while the cell was hyperpolarized. The EPSP evoked by the binaural signal is slightly larger than EPSP evoked by the contralateral signal alone, even though the ipsilateral tone presented alone evoked no response**. C:** EPSPs evoked by the contralateral tone and the binaural tone are superimposed. Tone duration, 5.0 ms.

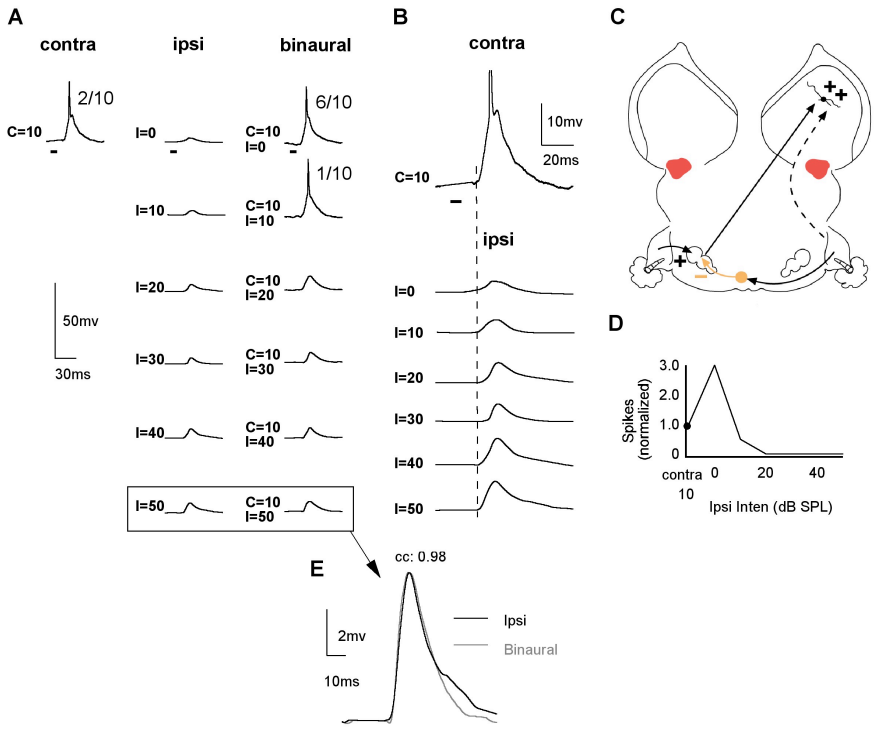

Fig 2.8. Facilitated EI cell (EI/f) in which ipsilateral tones evoked only EPSPs and facilitation was apparently produced by a summation of excitations evoked by ipsilateral and contralateral tones. Higher ipsilateral intensities evoked progressively larger EPSPs but suppressed discharges when presented binaurally. Panels A–E are same as in Fig. 2.4. Amplitude of ipsilateral EPSP in E is ~8.0 mV.

Binaural response properties at higher contralateral intensities

The binaural features shown in the previous sections were derived with low intensity contralateral tones that ranged from 10-30 dB SPL. Ten cells, 7 EI and 3 EI/f cells, were also evaluated with a second contralateral intensity that was 20-40 dB more intense than the lower intensity. In each of the ten cells, the same binaural property evoked with the lower contralateral intensity was also evoked at the higher intensity, which showed that the binaural features were independent of intensity. We illustrate the similar binaural properties at two intensities with the EI cell in Fig. 2.9.

The EI cell in Fig 2.9 is the same cell shown in Fig 2.4, but the contralateral intensity in Fig 2.4 was 10 dB SPL whereas the contralateral intensity in Fig 2.9 was 50 dB SPL. The circuit that may have generated the responses at 50 dB is shown in Fig 2.9B. The spike-count evoked by the 50 dB SPL contralateral tone was lower than the spike-count evoked by the 10 dB SPL contralateral tone because the cell's rate-intensity function was non-monotonic (not shown). Thus, the 50 dB SPL contralateral signal apparently evoked both an excitation and inhibition for reasons given below. In addition, the IID at which spikes were completely suppressed was slightly different with the 50 dB SPL contralateral tone (C=50, I=30 dB) than it was for the 10 dB contralateral tone (C=10, I=40 dB), presumably due to the smaller spike-count evoked by the 50 dB contralateral signal. In other respects, the binaural properties were similar. For example, whether the contralateral tone was 10 or 50 dB SPL, the PSPs evoked by binaural stimuli

with ipsilateral tones at 50 dB SPL were almost identical (Fig 2.9D). Additionally, those binaurally evoked PSPs were virtually the same as the PSPs evoked by 50 dB SPL ipsilateral signals presented alone.

The circuit that could explain the cell's response properties, incorporates an excitatory projection from the opposite LSO, an ipsilaterally evoked excitatory projection and an additional inhibitory input driven by high but not low contralateral intensities. Since LSO neurons have monotonic rate-intensity functions (Tsuchitani and Boudreau 1967; Tsuchitani and Johnson 1985; Park, Monsivais et al. 1997), the additional inhibitory input would account for the non-monotonic rate-intensity function of the IC cell. The nucleus that provided that inhibition was almost certainly binaural because there was no evidence of contralateral inhibition with the binaural tones at C=50, I=50 (Fig 2.9A). The contralateral inhibition may have come from the DNLL on the same side as the IC, since the DNLL is a binaural nucleus with EI properties (Brugge, Anderson et al. 1970; Yang and Pollak 1994; Kelly, Buckthought et al. 1998) that provides inhibitory projections bilaterally to EI cells in the IC (Adams and Mugnaini 1984; Shneiderman, Oliver et al. 1988; Ross and Pollak 1989; Shneiderman and Oliver 1989; Winer, Larue et al. 1995). We propose that the 50 dB contralateral signal drove two lower binaural inputs, excitation from the LSO and inhibition from the DNLL on the same side as the IC (Fig 2.9B). The ipsilateral signal drove three inputs; one that inhibited the LSO, one that inhibited the DNLL and a third input that evoked a subthreshold excitation. As the ipsilateral intensity was increased in the binaural signals,

the inputs from the ipsilateral ear progressively inhibited both the excitation from the LSO and the inhibition from the DNLL, while increasing the excitation to the IC. When the ipsilateral intensity was 40 or 50 dB SPL, both the excitation from the LSO and the inhibition from the DNLL were completely suppressed, leaving only the ipsilaterally evoked excitation. Such a circuit would account for the identity of the PSPs evoked by binaural signals with 50 dB SPL ipsilateral intensities with contralateral signals of 10 and 50 dB SPL, even though a higher intensity contralateral signal presented alone evoked both an excitation and inhibition.

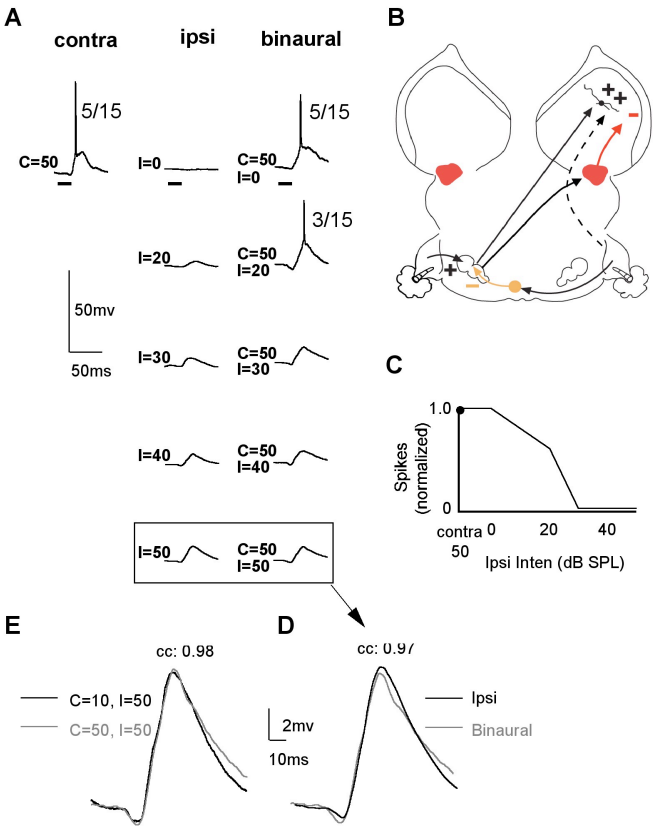

Fig 2.9. The same EI cell shown in Fig. 2.4 but the contralateral intensity was 50 dB SPL and thus the contralateral intensity was 40 dB more intense than was presented in Fig. 2.4. **A:** Responses to contralateral tones, ipsilateral tones and tones presented binaurally. **B:** The circuit that could generate the responses. The circuit is the same as in Fig. 2.4 with the addition of a high threshold, inhibitory input from the DNLL to account for the reduced spike count at 50 dB SPL, i.e., the upper-threshold rate level function. See text for further explanation**. C:** IID function based on normalized spike-counts. **D:** Responses to ipsilateral tone alone at 50 dB SPL and the binaural response evoked by binaural tones with the same ipsilateral intensity superimposed at higher magnification. **E:** Superimposed responses showing that virtually the same responses were evoked by binaural signals when contralateral signals were 10 or 50 dB SPL and ipsilateral intensity was 50 dB SPL. Tone duration, 20.0 ms.

DISCUSSION

This study showed five major features of EI and EI/f cells in the IC. The first is that a minority of EI cells inherited their response properties from a lower binaural nucleus. Those cells were in essence "monaural" in that they received excitatory inputs only from the contralateral ear and received no innervation from the ipsilateral ear. The second feature is that the EI property in a few cells was formed *de novo* in the IC via excitatory projections from the contralateral ear that were suppressed in the IC by inhibitory projections from the ipsilateral ear. The third, and most surprising feature, was that the majority of EI cells received excitatory inputs not only from the contralateral ear, but also from the ipsilateral ear, and in many of those cells, ipsilateral stimulation evoked only EPSPs. The fourth feature concerns EI/f cells, and is that EPSPs were evoked by ipsilateral stimulation at IIDs that evoked facilitation. Finally, the fifth feature is that in almost all EI/f cells, EPSPs were evoked by ipsilateral stimulation at IIDs that suppressed contralaterally evoked spikes, in ways identical to conventional EI cells. Below we discuss how these results compare to previous studies and then propose some functional consequences for the ipsilaterally evoked EPSPs that were so commonly observed in both EI and EI/f cells.

Comparisons with previous extracellular studies

Previous studies showed that EI and EI/f cells comprise a diverse subpopulation of the IC since each type is formed by different circuitry (Faingold, Gehlbach et al. 1989; Li and Kelly 1992; Faingold, Anderson et al. 1993; Park and Pollak 1993; Park and Pollak 1994). The circuits proposed in previous studies are shown in Fig 1.6A-C and invoked various combinations of three projections; 1) the projection of the LSO to the contralateral IC; 2) the projections of the DNLL to the contralateral IC; and 3) an excitatory projection of unknown source from a lower monaural nucleus that was activated by stimulation of the ear contralateral to the IC**.** Our results can, with the addition of a circuit for ipsilateral evoked EPSPs, be explained by the three projections and are largely in agreement with previous studies. Thus, previous extracellular studies reported that the EI properties of some cells were unchanged when inhibition was blocked or when the contralateral DNLL was reversibly inactivated (Fig 1.6A). Those cells are directly comparable to the EI cells we recorded in which ipsilateral stimulation evoked no subthreshold response at any intensity, and must have inherited their EI properties from the LSO. Previous studies also showed that the spike suppression in other EI cells was due to inhibitory projections from the opposite DNLL (Fig 1.6B). In those studies, spike suppression with binaural stimuli was abolished when the DNLL on the side opposite to the IC was inactivated, and the spike suppression then returned following inactivation (Li and Kelly 1992; Faingold, Anderson et al. 1993; Burger and

Pollak 2001). The changes in EI properties of the cells due to DNLL inactivation are strikingly similar to the EI cells in which contralateral stimulation evoked excitation and ipsilateral stimulation evoked IPSPs, where the amplitudes of the IPSPs increased with ipsilateral sound intensity (e.g., the cell in Fig 2.3).

The projection that was not proposed in previous studies is a subthreshold EPSP evoked by ipsilateral stimulation, since that response was undetectable with extracellular recordings even when inhibition was blocked. Indeed, it seems likely that some of the EI cells in previous extracellular studies that were unaffected when inhibition was blocked were cells that inherited their EI property from LSO projections but also had ipsilaterally evoked, subthreshold EPSPs. The EI properties would not change when inhibition was blocked because the EI property itself is first generated in the LSO, in the same way described above for cells that inherit their EI property but have no ipsilaterally evoked responses. The scenario for EI cells with EPSPs is that low intensities at the ipsilateral ear evoke no subthreshold responses, but with increasing ipsilateral intensities the LSO is progressively inhibited while the amplitude of the ipsilateral EPSP progressively grows. The ipsilaterally evoked EPSPs presumably add to the decreasing EPSPs from the LSO, which would adjust the IID at which the cell is completely inhibited so that higher intensities at the ipsilateral ear would be required to completely inhibit the cell than if the cell only received projections from the LSO. At IIDs with high ipsilateral intensities, the LSO is completely inhibited leaving only a subthreshold EPSP evoked by the ipsilateral ear, which would not be detected when inhibition was blocked because it was

subthreshold. This interpretation is supported by the EPSPs in both EI and EI/f cells evoked by binaural stimulation at IIDs with ipsilateral intensities 10-40 dB higher than the contralateral intensity. In all of these cells, the binaurally evoked EPSPs were virtually identical to the EPSPs evoked only by ipsilateral stimulation at the same intensity as in the binaural signals.

Other EI cells had ipsilaterally evoked inhibition and an ipsilaterally evoked subthreshold excitation that was activated at intensities higher than those that activated the inhibition (Fig 2.6). Their EI property is probably inherited from the LSO and is further shaped by an ipsilaterally evoked inhibition at low IIDs that may have originated from the opposite DNLL. These features are consistent with some cells in previous extracellular studies in which blocking GABAergic inhibition did not eliminate their EI property but rather changed the IID that caused a complete or nearly complete inhibition (Faingold, Gehlbach et al. 1989; Li and Kelly 1992; Park and Pollak 1993).

The same circuitry that we proposed for the EI cells with ipsilateral EPSPs could also account for the EI/f cells that expressed ipsilateral EPSPs at the IIDs that evoked facilitation. The only difference in the EI/f cells is that the circuit that generates the ipsilateral EPSPs has lower thresholds than the ipsilateral circuits in the conventional EI cells. In this scenario, binaural signals with low ipsilateral intensities would evoke both the excitation from the LSO and a small ipsilaterally evoked EPSP whose summation would generate a spike-count greater than that evoked only by the LSO excitation, and thereby evoke the facilitation.

Potential functional consequences of the ipsilaterally evoked EPSPs

What functional impact could the ipsilaterally evoked EPSPs have on binaural processing of sound for localization? One possibility is that their influence would be apparent only in more complex acoustic environments with moving sounds or when several sounds are received in succession. Consider, for example, a sound that moves around the head from the ipsilateral to the contralateral sound field. The initial EPSP could summate with the stronger excitation evoked as the sound moves into the contralateral field and thereby evoke a stronger discharge than would a stationary sound in the contralateral sound field. Another possibility applies to two or more sounds that followed each other within a short interval and that emanate from the same location in space, a location that generates an IID more intense at the ipsilateral ear. The first sound would evoke only a subthreshold EPSP, while the EPSP evoked by the following sound(s) would summate with the first EPSP. Assuming the summated response is suprathreshold, there would be a change in the responsiveness of the IC cell for the trailing sound(s) produced by the reception of an earlier sound whose IID only generates a subthreshold EPSP.

It is noteworthy that such a change in responsiveness to a trailing signal occurs in neurons whose EI properties are formed *de novo* in the IC through GABAergic projections from the opposite DNLL (Burger and Pollak 2001; Pollak, Burger et al. 2003; Pecka, Zahn et al. 2007). In those cells, initial signals with IIDs that are stronger in the

contralateral ear allow the cell to discharge to trailing signals with IIDs that, when presented alone, completely suppress discharges. Such changes in the binaural sensitivities of EI cells have been shown to contribute to the precedence effect, a percept common to all animals (Wyttenbach and Hoy 1993; Keller and Takahashi 1996; Burger and Pollak 2001; Keller and Takahashi 2005; Pecka, Zahn et al. 2007). The precedence effect is caused by a mechanism that suppresses the directional information carried by echoes (Wallach, Newman et al. 1949; Zurek 1987; Litovsky, Colburn et al. 1999; Pecka, Zahn et al. 2007). When initial and trailing sounds are presented, listeners hear a single composite sound and perceive the composite sound as originating from the leading speaker.

The difference between cells that express ipsilateral EPSPs and those that express inhibition to ipsilateral signals is that the EPSPs could change the responsiveness to signals that emanate from the same locations in space whereas cells that express ipsilateral inhibition only change the responsiveness to signals that emanate from different regions of space. Together, the two types of cells could respond to trailing signals from any region of space while degrading the code for the location of the trailing sound. Previous studies suggested that dynamic IIDs generate different responses in IC cells than do static IIDs (Sanes, Malone et al. 1998; Burger and Pollak 2001). Given the prevalence of ipsilaterally evoked EPSPs among EI cells, features that change binaural processing in dynamic acoustic environments may be more prevalent than previously suspected.

Caveat of predicting circuitry based on PSPs

Intracellular recordings don't always provide accurate information about the inputs. For example, when we see no PSP is evoked, it is not really sure whether any inputs are evoked because of the following reasons. It is possible that the resting potential is close to the reversal potential of Cl channels so the driving force for Cl is too small, thus no change of membrane potential is evoked. It is also possible that inhibition and excitation are coincident in time and cancelled out each other, therefore producing no change of membrane potential.

In order to really measure the inputs, we need to separate the excitatory inputs from inhibitory inputs. One way to do this is to calculate synaptic conductances. Conductance measurement will allow me to separate excitatory inputs and inhibitory inputs, and will provide me the information about timing and magnitude of excitatory inputs and inhibitory inputs. In addition, conductances evoked by different inputs can sum linearly. Conductance analysis will be discussed in the next chapter.

CHAPTER 3: *CONDUCTANCE ANALYSIS REVEALS THE CIRCUITS OF THE NEURONS THAT CODE IID*

INTRODUCTION

In the previous chapter I showed that most of the cells recorded in the IC with in vivo whole patch recordings are binaural and exhibit EI properties. I also showed that EI cells in the IC comprise a diverse group, even though they exhibit binaural response properties similar to LSO cells. Some EI cells inherit their full binaural properties from a lower nucleus, presumably the LSO, in others their binaural properties are only partially inherited, since they receive excitatory innervation from the ipsilateral ear, and in others the EI property is created in the IC through excitatory projections from the contralateral ear and inhibitory projections from the ipsilateral ear. All of these features were inferred from the postsynaptic potentials (PSPs) evoked by monaural stimulation of the contra- and ipsilateral ears and by binaural stimulation.

Here I extend the evaluations of the various ways by which EI neurons are formed by computing the excitatory and inhibitory conductances evoked by stimulation of each ear alone and by binaural stimulation. Conductance analysis not only allow me to separate excitatory from inhibitory inputs, they also provide information about the timing

and magnitude of the excitatory and inhibitory inputs. Additionally, unlike PSPs, conductances sum linearly. The linear summation allows the excitatory (or inhibitory) conductances evoked by monaural stimulation of the contralateral and ipsilateral ears to be added, and the summed conductances can be compared to the excitatory (or inhibitory) conductance evoked by binaural stimulation. If the summed excitatory (or inhibitory) conductances evoked by each ear are equal to the binaural excitatory conductance, I conclude that the excitation evoked by the contralateral and ipsilateral ears are projected separately to the IC where they summate linearly. If the summation of the monaural conductances is not equal to the binaural conductance, I conclude that the inputs from each ear interacted in a lower nucleus and their influence on the IC is not equal to the linear addition of their effects when each is presented only to one ear. The same logic applies to the inhibitory conductances.

The general strategy for the assessment of conductances is illustrated by the hypothetical EI neuron in Fig 3.1, a neuron whose EI property is formed by an excitatory projection evoked by the contralateral ear and an ipsilaterally evoked inhibition. In this hypothetical example, a 10 dB SPL tone at the contralateral ear evokes discharges that are suppressed by ipsilateral tones, where complete spike suppression first occurs at 30 dB SPL. The calculated conductances show that a strong excitatory conductance is evoked by a10 dB contralateral tone, no excitatory conductance was evoked by an ipsilateral tone at 30 dB, and that the excitatory conductance evoked by binaural stimulation at the same intensities, contra=10dB SPL, ipsi=30dB SPL, was identical to

the excitatory conductance evoked by the contralateral signal alone. Since the excitatory conductance was not influenced by binaural stimulation, excitatory projections from the contralateral ear must have originated from one or more lower monaural nuclei that were innervated only by the contralateral ear.

The inhibitory conductances, in contrast, show that the ipsilaterally evoked inhibition must have originated in a lower binaural nucleus, most likely the opposite DNLL. The reason is that contralateral stimulation evoked no inhibitory conductance whereas ipsilateral stimulation evoked a large inhibitory conductance. However, the inhibitory conductance evoked by binaural stimulation was smaller than the inhibition evoked when the ipsilateral ear were stimulated alone. The difference showed that the inhibition evoked by the ipsilateral ear was reduced by the introduction of a contralateral signal, and thus the inhibition must have originated from a lower binaural nucleus, which by definition is innervated by both ears.

As a final check, I can work backwards and calculate the PSP that should have been evoked by the binaural excitatory and the binaural inhibitory conductances. If the conductance calculations are indeed correct, then the calculated binaural PSP should provide an accurate prediction of the PSP evoked by sound at that IID. I validate the accuracy of each set of conductance derived from monaural or binaural stimulation. Thus by comparing conductances evoked by monaural and binaural stimulation a more detailed view that will either support the hypotheses that were proposed in the previous section, or

will require additional inputs that were not revealed by the evaluation of monaurally and binaurally evoked PSPs.

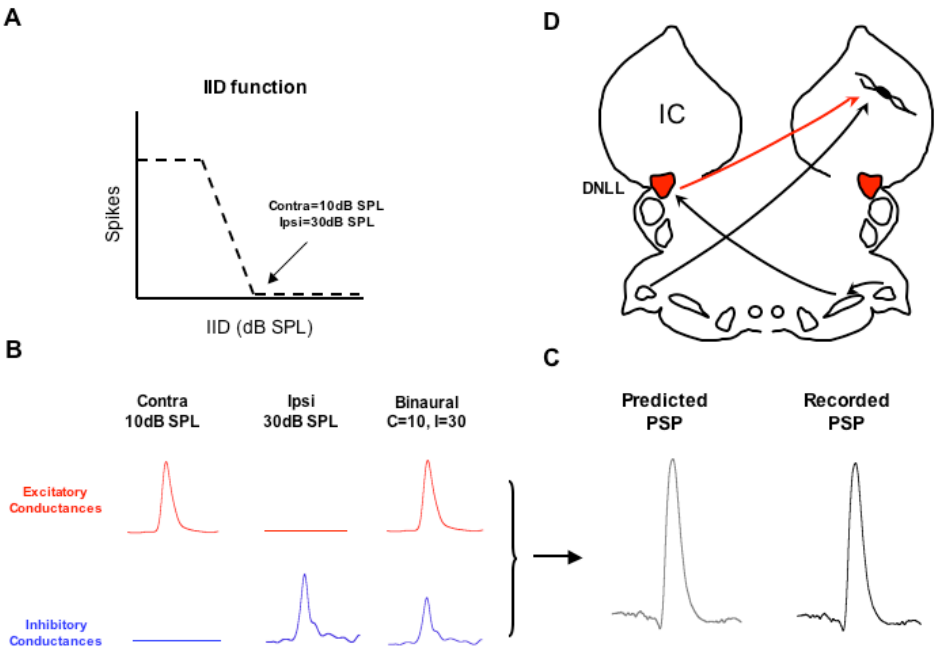

Fig 3.1. A schematic illustration of my strategy for the assessment of conductances. **A:** The IID function of an EI cell. Conductances were derived at an IID of 20dB SPL (contra=10dB SPL, ipsi=30 dB SPL) where all spikes were suppressed with binaural signals. **B:** Top panel: a large excitatory conductance was evoked by the contralateral signal and no excitatory conductance was evoked by the ipsilateral tone. The binaural conductance was the summation of the contra- and ipsilateral excitatory conductances. Bottom panel: no inhibitory conductance was evoked by the contralateral signal and a large inhibitory conductance was evoked by the ipsilateral signal. The binaural conductance was smaller than the summation of the contra- and the ipsilateral inhibitory conductances. **C:** PSPs were predicted by using derived conductances. Conductances were validated by a good correlation between predicted PSPs and measured PSPs. **D:** Circuit predicted based on conductance.

METHODS

Surgical Procedures, Acoustic stimuli, Recording Procedures and Data Acquisition

The surgical procedures, acoustic stimuli, recording procedures and data acquisition are same with chapter 2. If we got substantial time periods and stable recording, we will perform conductance measurements. We recorded PSPs and spikes evoked by 15-20 tone presentations while hyperpolarizing the cell with at least three different steady state current injections. The contralateral tone was normally played at one intensity which is 10-20 dB above the threshold. The ipsilateral tone was normally played at one or two intensities with which a binaural stimulation can substantially affect the contralateral responses. The conductance was computed individually for contralateral stimulation, ipsilateral stimulation, and binaural stimulation.

Estimating access resistance, membrane resistance and membrane capacitance

The electrode capacitance was minimized by doing online capacity compensation. The access resistance, membrane resistance, membrane capacitance and membrane time constant were estimated by fitting voltage responses to small hyperpolarizing current steps (25 – 100 pA, 200 ms duration) with a double exponential fit (equation 1) as in previous studies (Priebe and Ferster 2005; Gittelman, Li et al. 2009) (Fig 3.2):

$$V_t = V_p*(1-\exp(-t/\tau_p)) + V_m*(1-\exp(-t/\tau_m)) \quad (1)$$

V_t was the measured change in voltage (total change) in response to the current injection. V_p was the steady state voltage change attributed to the pipette, and V_m was the steady state voltage change attributed to the membrane. The fast and slow time constants were attributed to the pipette (τ_p) and membrane (τ_m) respectively. Membrane resistance (R_m) was equal to the change in membrane voltage divided by the injected current ($R_m = V_m/I_{inj}$). Membrane capacitance (C_m) was then calculated as τ_m divided by R_m ($C_m = \tau_m \div R_m$). Access resistance and electrode capacitance were estimated in a similar way, using the fast components of the fit (V_p and τ_p). In recordings judged acceptable, the fast time constant < 1 ms and the associated access resistance was less than the estimated membrane resistance.

Estimating synaptic conductances

Synaptic conductances were estimated as in previous studies (Priebe and Ferster 2005; Gittelman, Li et al. 2009) by using the equation:

$$C * dV_m/dt = -\Sigma I_{membrane} + I_{inject} \qquad (2)$$

where C is the cell capacitance, dV_m/dt is the slope of the membrane potential, $I_{membrane}$ is the current across the cell membrane, and I_{inject} is the current injected through the electrode. We assumed three sources of membrane current: an excitatory current, an inhibitory current, and a leakage current. Equation 2 can be expanded to include the conductance and driving force terms:

$$C * dV_m/dt = - [g_e (V_m-V_e) + g_i (V_m-V_i) + g_{leak} (V_m-V_{leak})] + I_{inject} \quad (3)$$

The conductances (g) are: excitatory, g_e; inhibitory, g_i; leak, g_{Leak}. V_m is the measured membrane potential, and the reversal potentials for g_{leak}, g_e and g_i are (respectively) V_{leak}, V_e, and V_i. Most of these terms can be measured or estimated. V_m and dV_m/dt were measured directly. Capacitance and input resistance ($1/g_{leak}$) were measured as described above. V_e was assumed to be 0 mV, and V_i was estimated to be – 68 mV from the changes in the PSP polarity while different amounts of constant current were being injected. V_{leak} was resting potential when no current was injected through the electrode. When the cell was hyperpolarized, V_{leak} was calculated from the steady state V_m, the input resistance measured at the steady state V_m, and the injected current. When the cell was hyperpolarized, V_{leak} was typically depolarized with respect to resting potential.

Using the above values, there are only two unknowns in equation 3, g_e and g_i. Consequently, conductances can be estimated from sound evoked responses while hyperpolarizing the cell to only two different steady state potentials. In practice, we required sound evoked responses recorded while hyperpolarizing to at least three different steady state membrane potentials.

Modeling

Model cells were 'point model cells', consisting only of excitatory, inhibitory and leak conductances with corresponding reversal potentials and a capacitance. We made a

unique model cell for each neuron used in the conductance estimates based on the measured input resistance, resting membrane potential and capacitance in each cell. For cells where we estimated conductances from the responses to three tone sets (contra-, ipsi-, and binaural), the same model was used for all three conductance sets. (Fig 3.2)

The validity of the conductance estimates was determined by two criteria. We used the estimated values for g_e and g_i to predict the voltage responses in the models. That predictions were good with an average correlation coefficient (>0.9) across a broad range of membrane potentials suggests that the state of voltage-gated channels changed little during the time course of the PSPs. Second, we excluded analyses that found negative values for conductance. This requirement assumed that the ligand-gated channels were closed (0 nS) prior to sound presentation, and thus the conductances could only get larger. This is a reasonable assumption because there was little or no spontaneous activity in the IC cells. The absence or near absence of spontaneous activity is a common finding in the IC of bats (Klug, Bauer et al. 1999; Bauer, Klug et al. 2000).

RESULTS

I derived synaptic conductances from 6 cells in the central nucleus of the inferior colliculus (ICc) of awake bats. For each neuron, I derived 3 sets of synaptic conductances; one evoked by a contralateral tone, another evoked by the ipsilateral tone

and a third conductance evoked by binaural tones that had the same contra and ipsilateral intensities as monaural tones. If time allowed, I derived another sets of synaptic conductances at a different ipsilateral intensity and thus at a different binaural IID. Five cells were conventional EI cells and one cell was an EI/f cell. Among the five EI cells, one cell displayed ipsilaterally evoked hyperpolarization while four others had ipsilaterally evoked depolarization.

Conductances evoked in an EI cell that has ipsilaterally evoked inhibition

I first consider the EI cell in which contralateral stimulation evoked excitation and discharges while ipsilateral stimulation only evoked IPSPs. This cell is similar to the cell shown in Fig 2.3 in Chapter 2 in the following ways. First, the amplitudes of the IPSPs increased with ipsilateral sound intensity. Second, with binaural stimulation, the contralaterally evoked excitation was progressively reduced as the intensity at the ipsilateral ear increased, suggesting that the ipsilateral IPSPs, presumably from contralateral DNLL, inhibited the contralateral excitation and formed the cell's EI property de novo in the IC. If this suggestion is correct, the conductances should correspond to those shown for the model cell shown in Fig 1.6B. Specifically, the excitatory conductance evoked by the contralateral ear should not be affected by introducing a tone to the ipsilateral ear, whereas the inhibitory conductance evoked by the ipsilateral ear should be influenced by binaural stimulation.

The conductances were derived from the responses evoked by monaural stimulation of each ear and by binaural stimulation at an IID of 10 dB (Contra=30dB, Ipsi=20dB). Binaural tones at this IID caused about an 80% reduction of spike count evoked by the contralateral stimulus (Fig 3.3). In the sections below, I first consider the excitatory conductances and then turn to the inhibitory conductances.

Comparing excitatory conductances showed that the excitatory conductance evoked by the contralateral ear was influenced by stimulation to the ipsilateral ear, and thus did not confirm the expected outcome (Fig 3.4B). There are two key features. First, monaural stimulation of the ipsilateral ear did not evoke an excitatory conductance (Fig 3.4B, Ipsi). Second, the excitatory conductance evoked by binaural stimulation was smaller than the excitatory conductance evoked only by stimulation of the contralateral ear (Fig 3.4C). This suggests that the excitation at the IC originated at least in part from a binaural nucleus, presumably the LSO. The reasoning is that contralateral stimulation should excite the LSO and thereby evoke a large excitatory conductance. However, introducing a tone at the ipsilateral ear would partially suppress the LSO output, thereby generating a smaller excitation with binaural than with monaural stimulation at the IC.

Unlike the contralaterally evoked excitatory conductance, the ipsilaterally evoked inhibitory conductance was in agreement with the model prediction, in that it originated from a lower binaural nucleus, presumably the DNLL. There are two noteworthy features. First, monaural stimulation of the contralateral ear evoked a small inhibitory conductance (Fig 3.4D, Contra). Second, a large inhibitory conductance was evoked by

monaural ipsilateral stimulation at 20 dB (Fig 3.4D, Ipsi) whereas the inhibitory conductance is smaller or reduced with binaural stimulation (Fig 3.4F). The inhibitory conductance with binaural stimulation had a similar amplitude as the inhibitory conductance with ipsilateral stimulation, but the shape of the inhibitory conductance with binaural stimulation was narrower (Fig 3.4F). Therefore the total conductance, the area under the waveform, was smaller with binaural than that evoked by ipsilateral stimulation alone. When I summed the inhibitory conductances evoked by the contra- and ipsi-stimulation and compared the summation with the binaural inhibitory conductance, the binaural conductance was not only narrower but also smaller in magnitude than the summed inhibitory conductance (Fig 3.4E). This suggests that the inhibition at the IC originated at least in part from a binaural nucleus, the DNLL (Fig 3.4G). The reasoning is that ipsilateral stimulation should excite the DNLL and thereby evoke a large inhibitory conductance at the IC. However, introducing a tone at the contralateral ear would partially suppress the DNLL output, thereby generating a smaller inhibition with binaural than with monaural stimulation at the IC. This inhibitory innervation from DNLL is consistent with the model suggested by previous extracellular studies.

Finally, I show that the reduction in the excitatory conductance with binaural stimulation was necessary to achieve the results with binaural stimulation. To evaluate the importance of the reduction in the excitatory conductance in shaping the EI property, I manipulated the binaural excitatory conductance to make its total conductance equal to the total conductance of contralateral excitatory conductance (Fig 3.5A). Then I injected

this manipulated binaural excitatory conductance and the original binaural inhibitory conductance into a point model cell to predict the manipulated binaural response (PSP); the PSP that would have been evoked if the excitatory conductance had been evoked by a monaural, excitatory input. The manipulated binaural response was larger than the control binaural response and substantially above the spike threshold (Fig 3.5B). The large suprathreshold response suggested that if there were no reduction of excitation with binaural stimuli, the binaural stimulus would have evoked a high spike count that was only slightly smaller than the spike count evoked when the tone was presented monaurally to the contralateral ear. Thus the reduction in the excitatory conductance with binaural stimulation was necessary to achieve the great suppression of spikes that was evoked with binaural stimulation.

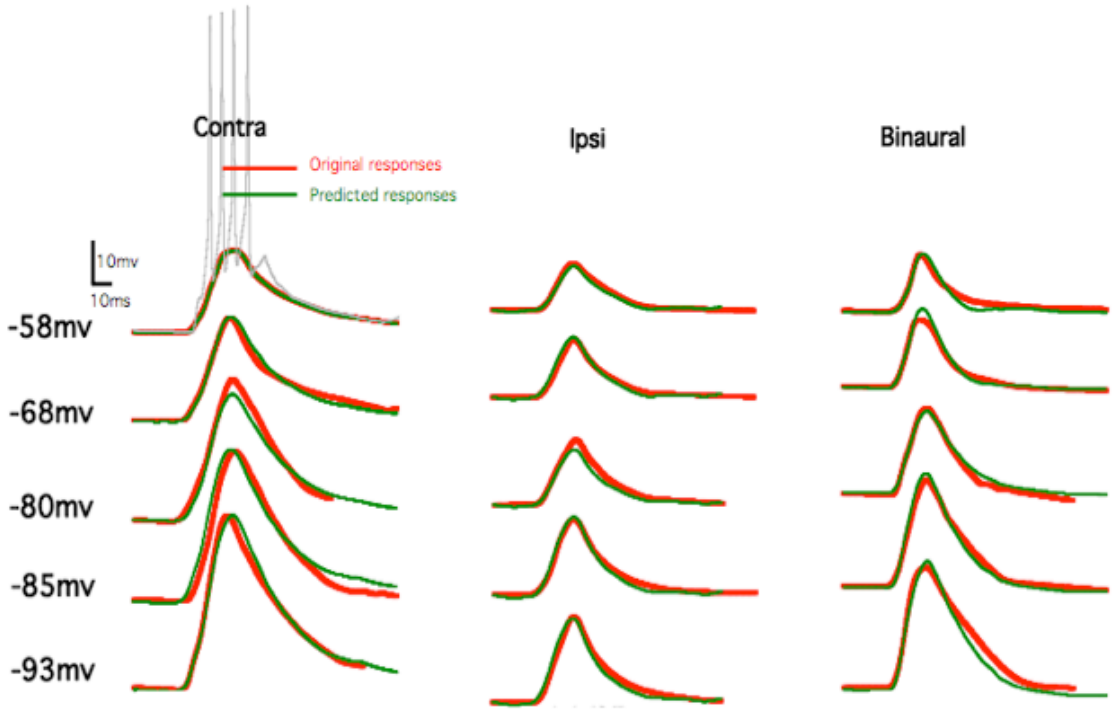

Fig 3.2. Recorded responses (red) of an EI cell with different current holding current and predicted responses based on derived conductances (green). With each hold current, same contra-, ipsi- and binaural stimuli were played and another set of sound evoked responses were recorded.

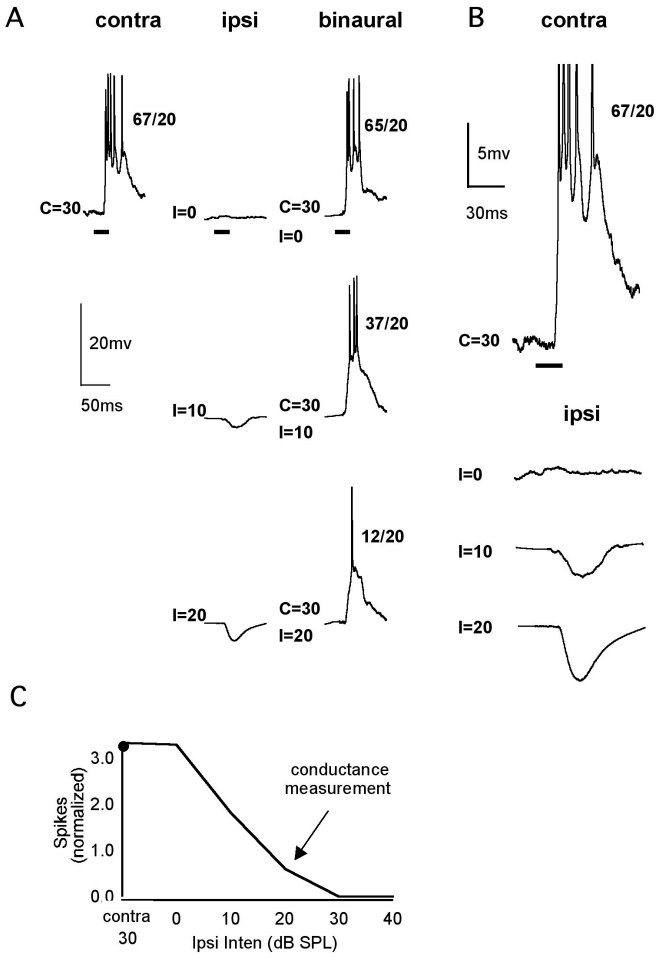

Fig 3.3. Responses of an EI cell in which ipsilateral signals only evoked IPSPs. **A:** An EI cell in which ipsilateral signals only evoked IPSPs that increased with intensity. With binaural signals, the contralateraly evoked spikes were progressively suppressed as ipsilateral sound intensity was increased. The spike suppression mirrored the increase in the IPSP amplitude with ipsilateral intensity. **B:** Excitatory response to a 30 dB SPL contralateral tone aligned with IPSPs evoked by ipsilateral tones at different intensities showing that the latencies of the IPSPs were coincident with the discharges evoked by a contralateral tone. **C:** IID function. Arrow showed the IID where conductances were derived.

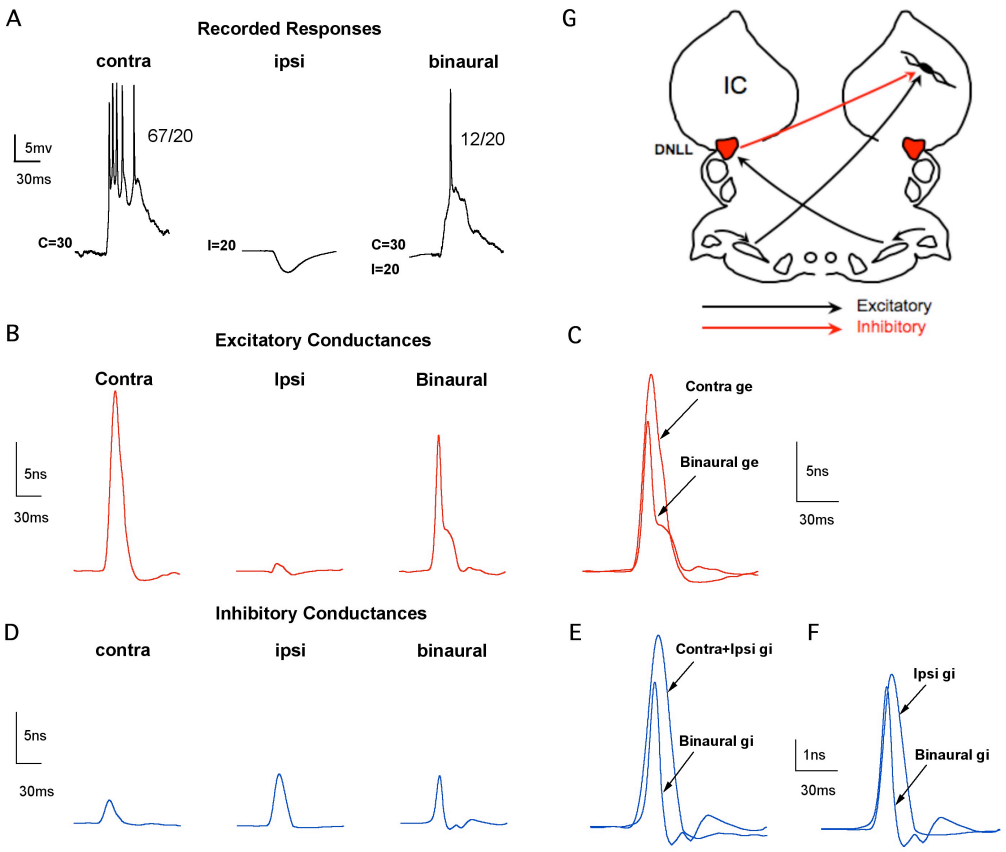

Fig 3.4. Conductances derived from the EI cell shown in Fig 3.2. **A:** Recorded PSPs and spikes evoked by a contralateral tone (30dB SPL), an ipsilateral tone (20dB SPL), and binaural tones (Contra=30dB SPL and Ipsi=20dB SPL). **B:** Excitatory conductances derived. **C:** A comparison between contra- and binaural excitatory conductance. **D:** Inhibitory conductances derived. **E:** A comparison between binaural excitatory conductance and the summation of contra- and ipsilateral excitatory conductances. **F:** A comparison between ipsi- and binaural excitatory conductance. **G:** Predicted circuit. Contralateral inhibitory inputs were not shown due to a small contralateral inhibitory conductance.

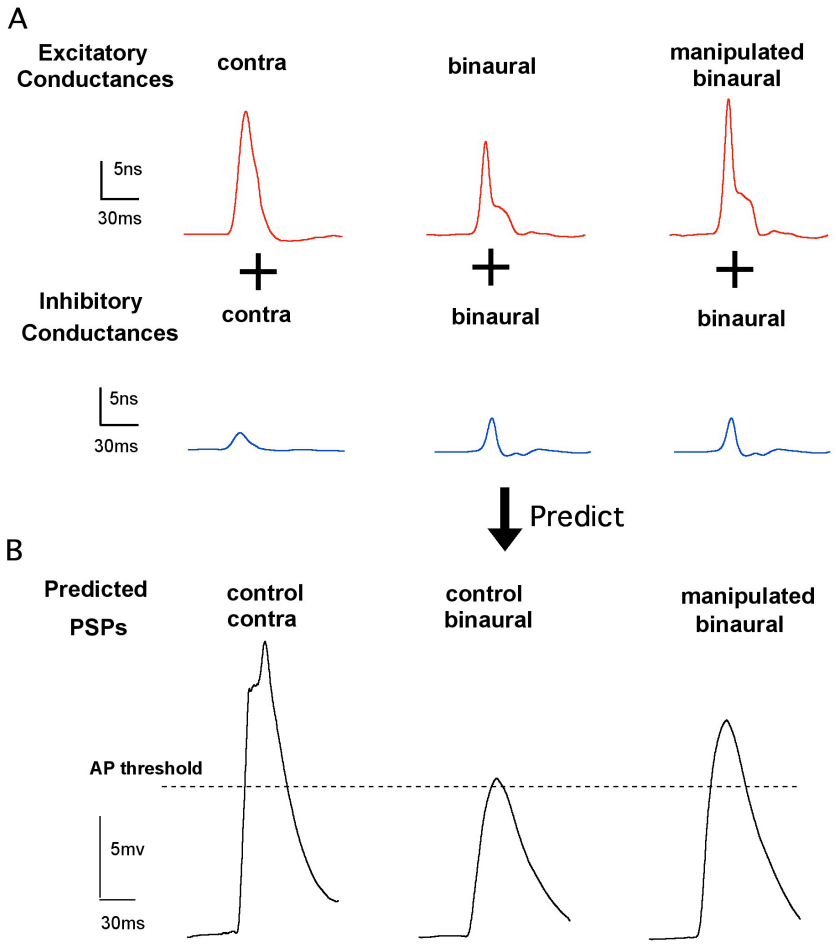

Fig 3.5. Reduction of binaural excitation was important for spike suppression. **A:** Conductances used to compute the predicted response. The manipulated binaural excitatory conductance was scaled so that it kept the waveform of the derived binaural excitatory conductance but the total area under the curve was equal to the derived contralateral excitatory conductance. **B:** Predicted responses with control contralateral conductances, control binaural conductances, and manipulated binaural conductances. The predicted response with manipulated binaural conductances was larger than the recorded binaural response, meaning producing a less spike suppression if there was no reduction of binaural excitation.

Conductances evoked in EI cells where ipsilateral tones only evoked EPSPs

The other EI cells (4/5 EI cells) from which I derived conductances either had only ipsilaterally evoked EPSPs whose amplitudes increased with ipsilateral intensities (Fig 3.6) or ipsilaterally evoked IPSPs that then changed into EPSPs at higher ipsilateral intensities (Fig 3.8). These were the most common type of EI cell in my sample. Based on their PSPs a working model was proposed in the previous section for EI cells that had only ipsilaterally evoked EPSPs. The model proposed that ipsilateral signals activated two projections: 1) one projection inhibits the contralateral LSO and inhibits contralaterally driven excitation; and 2) a second excitatory projection provides the ipsilateral excitation. In these cells, there was no evidence of hyperpolarization, suggesting that no local inhibition was evoked by contralateral and binaural tones. Additionally, binaural signals evoked subthreshold EPSPs at IIDs that produced a complete spike suppression, and those EPSPs were virtually identical to the EPSPs evoked by ipsilateral signals that had the same intensity as ipsilateral signal presented binaurally. I therefore proposed that the mechanism for generating the EI property, the spike suppression with increasing ipsilateral intensity, is by inheritance, through a reduction of contralateral excitation at a lower binaural nucleus, presumably the LSO, which provides the excitatory drive to the IC. The ipsilateral signal not only inhibited the LSO, and therefore suppressed all spikes at the IC, but through a second projection also evoked a subthreshold EPSP at the IC.

Evaluations of the conductances evoked by monaural and binaural tones revealed that the model outlined above, which as based only on the PSPs, is oversimplified, and that both excitation and inhibition were evoked by contra- and ipsilateral inputs. Moreover, the excitation and inhibition evoked by each ear act non-linearly in the IC, and that innervation from both contra and ipsilateral stimulation act mainly through lower binaural nuclei.

I illustrate these features with the EI cell in Fig 3.6. The panels in Fig 3.7 show both the PSPs (Fig 3.7A) evoked by monaural and binaural tones and the excitatory and inhibitory conductances (Fig 3.7B~I). In this cell, ipsilateral tones evoked EPSPs across all intensities (Fig 3.6A, B). The conductances were computed from monaural responses when the contra signal was 20 dB SPL, when the ipsi signal was 40 dB SPL, and from binaural signals presented at the same intensities. Contralateral tones at 20 dB evoked 7 spikes with 7 sound repetitions, whereas ipsilateral tones only evoked a subthreshold EPSP. Binaural tones at these intensities caused complete spike suppression and a subthreshold EPSP that was virtually the same as the ipsilaterally evoked EPSP.

Conductances computed from responses evoked by contralateral stimulation show that both excitatory and inhibitory conductances were evoked by contralateral stimulation (Fig 3.7B, F, contra). This was not surprising since blocking inhibition in extracellular studies almost always resulted in an increased spike count to contralateral stimulation. The unexpected features were the conductances evoked by ipsilateral and binaural stimulation. Ipsilateral stimulation, which only evoked an EPSP, evoked both an

excitatory and inhibitory conductance (Fig 3.7B, F, ipsi). Binaural stimulation at C=20 dB, I=40 dB also evoked both excitatory and inhibitory conductances (Fig 3.7B, F, binaural). However, the conductances evoked by binaural stimulation were not linear summations of the conductances evoked by monaural stimulation of each ear. Below I discuss how the excitatory and inhibitory conductances evoked by each ear interact to create the features of the binaurally evoked PSP.

I first consider the excitatory conductances. The binaural excitatory conductance was smaller than the summation of the contra and ipsi excitatory conductances (Fig 3.7C). This was expected because the model presented above predicted that the binaural excitatory conductance should be much smaller than the summation of excitation evoked by each ear and should be equal to the excitatory conductance evoked only by the ipsi ear. What was surprising is that the binaural excitatory conductance was larger than the ipsi excitatory conductance (Fig 3.7E). The larger binaural excitatory conductance requires that the contralateral excitation was not completely suppressed at this IID, but rather that there was still some residual contra excitation that summated with the ipsi excitation. This raises the question of how is it possible that a binaural excitation larger than the ipsilateral excitation can both generate virtually the same EPSPs?

The answer to this paradox resides in the behavior of the inhibitory conductances. Contralateral stimulation evoked a large inhibitory conductance whereas ipsilateral stimulation evoked only a small inhibitory conductance (Fig 3.7F). The inhibition evoked by the contralateral ear was greatly reduced with binaural stimulation

(Fig 3.7H), suggesting that the inhibitory projection was from a lower binaural nucleus that had EI properties, presumably the DNLL ipsilateral to the IC. Most importantly, the binaural inhibitory conductance was substantially larger than the inhibition evoked by the ipsilateral tone alone (Fig 3.7I). This suggests that the introduction of a signal at the ipsilateral ear reduced the inhibition evoked by the contralateral ear, but the residual contralaterally evoked inhibition summated with the ipsilateral inhibition to generate the binaural inhibition.

When considered together with the binaurally evoked excitation, the excitation evoked by the binaural signal may have produced a suprathreshold EPSP, but the binaurally evoked inhibition suppressed the excitation and together generated an EPSP that was virtually identical to the EPSP evoked by the ipsilateral signal alone.

In summary, the original hypothesis was based on the behavior of PSPs and proposed that with an IID of 20 dB, ipsilateral ear more intense, complete spike suppression occurred in the LSO and that the ipsilateral tone not only generated the inhibition at the LSO but through an independent projection, evoked a subthreshold EPSP. The evaluation of conductances suggests a more complex circuit was activated by contralateral stimulation (Fig 3.7J). Ipsilateral circuitry is only slightly more complex in that ipsilateral stimulation also activated both an excitation and inhibition that were from monaural nuclei and were not influenced by the contralateral ear. The hypothesis is that contralateral tones evoked both an excitation from the LSO and an inhibition from the DNLL on the same side as the IC. The ipsilateral projections inhibited the LSO, but not

completely thereby leaving a residual contralateral excitation with binaural stimulation. Ipsilateral stimulation also partially inhibited the DNLL and thus the inhibition evoked with binaural stimulation was less than that evoked by monaural stimulation of the contralateral ear. The residual contralaterally evoked excitation from the LSO summated with the excitation evoked by the ipsilateral ear but was suppressed to subthreshold levels by the remaining contralateral inhibition from the DNLL. Thus the EI property was not completely inherited from the LSO but was shaped by complex interactions in the IC by excitation and inhibition.

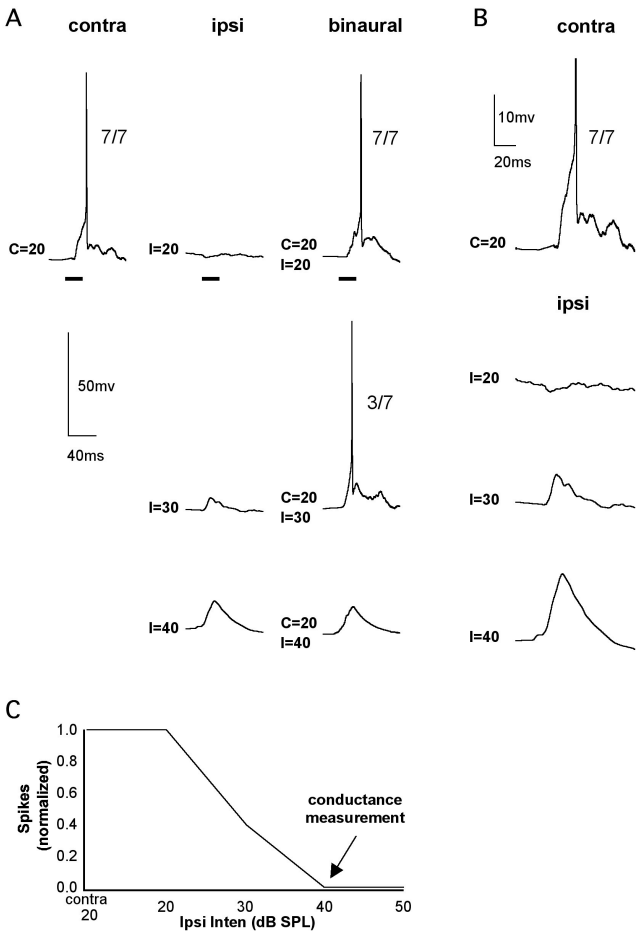

Fig 3.6. Responses of an EI cell in which ipsilateral signals only evoked EPSPs. **A:** An EI cell in which ipsilateral signals evoked subthreshold EPSPs that increased with intensity. With binaural signals, the contralaterally evoked spikes were progressively suppressed as ipsilateral sound intensity was increased. The spike suppression is paradoxical with the increases in the EPSP amplitude with ipsilateral intensity. **B:** Excitatory response to a 20 dB SPL contralateral tone aligned with EPSPs evoked by ipsilateral tones at different intensities showing that the latencies of the EPSPs overlaped with the response evoked by the contralateral tone. **C:** IID function. The arrow showed conductances were derived at an IID of 20dB SPL (Contra=20dB SPL and Ipsi=40 dB SPL).

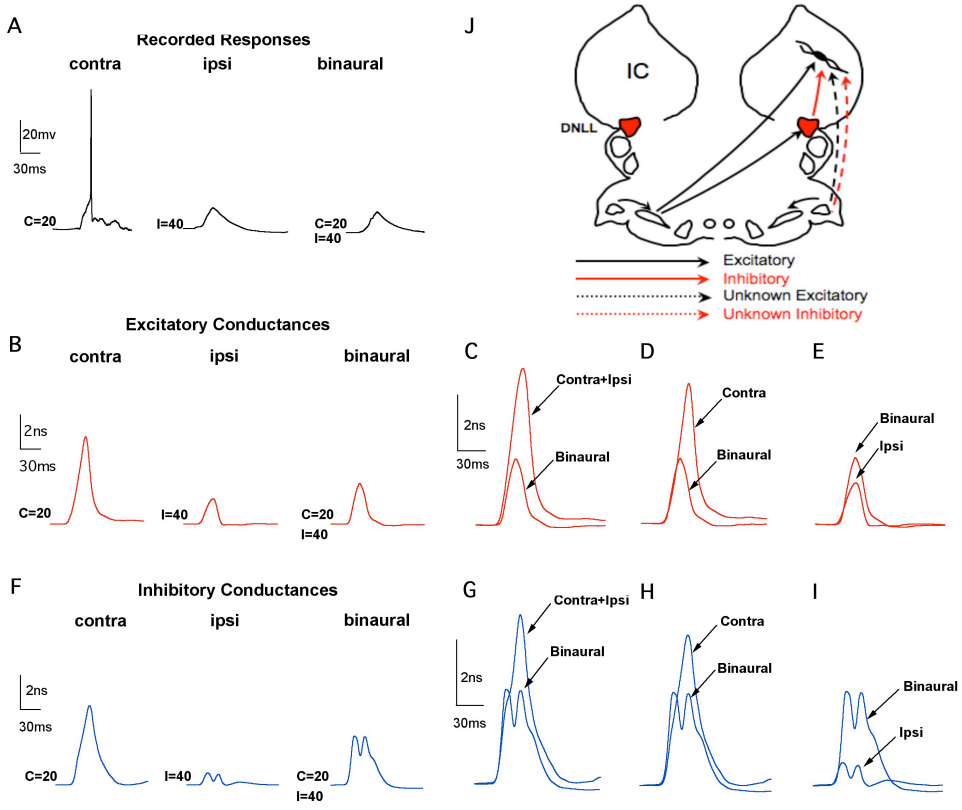

Fig 3.7. Conductances derived from the EI cell in Fig 3.5. **A:** Recorded PSPs and spikes with a contralateral tone at 20dB SPL, an ipsilateral tone at 40dB SPL, and binaural tones (Contra=20dB SPL and Ipsi=40 dB SPL). **B:** Excitatory conductances derived. **C:** A comparison between binaural excitatory conductance and the summation of contra- and ipsilateral excitatory conductances. **D:** A comparison between binaural and contralateral excitatory conductance. **E:** A comparison between binaural and ipsilateral excitatory conductance. **F:** Inhibitory conductances derived. **G:** A comparison between binaural inhibitory conductance and the summation of contra- and ipsilateral inhibitory conductances. **H:** A comparison between binaural and contralateral inhibitory conductance. **I:** A comparison between binaural and ipsilateral inhibitory conductance. **J:** Predicted circuit.

Conductances evoked in EI cells where ipsilateral tones evoked IPSPs at low intensities and EPSPs at higher intensities

The circuitry for EI cells in which ipsilateral signals evoked IPSPs at low intensities that then changed into EPSPs at higher intensities is similar to the EI cells in described above but requires that the ipsilaterally evoked inhibition, as well as the contralaterally evoked inhibition, originating from lower binaural inhibitory nuclei. We suggest that the lower binaural inhibitory nucleus that provides the contralaterally evoked inhibition is the DNLL on the same side as the IC, as in the previous cell, and that the ipsilateral inhibition is provided by the DNLL on the side opposite to the IC. The monaural and binaural PSPs for one of these EI neurons is shown in Fig 3.8 and the conductances are shown in Fig 3.9. The logic for the proposed circuit follows from the behavior of the inhibitory conductances.

Before discussing the inhibitory conductances, I show that the excitatory conductances are similar to the previous cell in Fig 3.7B. The first point to be made is that contralateral tones at 30 dB evoked a large excitatory conductance and ipsilateral tones at 50 dB evoked a smaller excitatory conductance, which generated the ipsilaterally evoked EPSP (Fig 3.9B). As with the previous cell, binaural stimulation at the same intensities as the monaural signals evoked an excitatory conductance that was smaller than the linear summation of the contra- and ipsilateral excitatory conductances (Fig 3.9C), suggesting a reduction in excitation due to inhibition at the LSO with binaural

stimulation. The binaural excitatory conductance, however, was larger than the ipsilateral excitatory conductance (Fig 3.9E), suggesting that the LSO was not completely inhibited by the IID at which the binaural tone was presented. The residual contralateral excitation summed with the ipsilateral excitation thereby evoking a larger binaural excitatory conductance than evoked by ipsilateral tones.

While the excitatory conductances were similar with the previous cell, the inhibitory conductances were slightly different. As seen in Fig 3.7F, contralateral stimulation evoked the largest inhibitory conductance, ipsilateral stimulation evoked a smaller inhibitory conductance and binaural stimulation evoked the smallest inhibitory conductance. The difference between this and the previous cell is that in the previous cell, the binaural inhibitory conductance was larger than the ipsilateral conductance (Fig 3.7I) whereas in this cell it was smaller (Fig 3.9I). Since the binaural inhibitory conductance was smaller than ipsilateral inhibitory conductance, the inhibitory conductance evoked by the ipsilateral ear was reduced by stimulation of the contralateral ear, and thus the inhibition must have originated from a lower binaural nucleus, e.g., the opposite DNLL that is activated by stimulation of the contralateral ear. The same argument applies to the contralaterally evoked inhibitory conductance that most likely originated from the other DNLL, on the same side as the IC (Fig 3.9J).

Finally, this all spins together so the binaural signal evokes a subthreshold EPSP that is slightly larger than the ipsilaterally evoked EPSP. As we described before, binaural signal generated an excitatory conductance that was larger than the excitatory

conductance evoked by the ipsilateral signal (Fig 3.9E). That excitatory conductance, if unopposed by inhibition, may have evoked a suprathreshold EPSP. However, the small binaurally evoked inhibitory conductance was sufficient to reduce the excitation to a subthreshold level, thereby generating a subthreshold EPSP but one that was just slightly larger than the EPSP evoked by the ipsilateral signal alone.

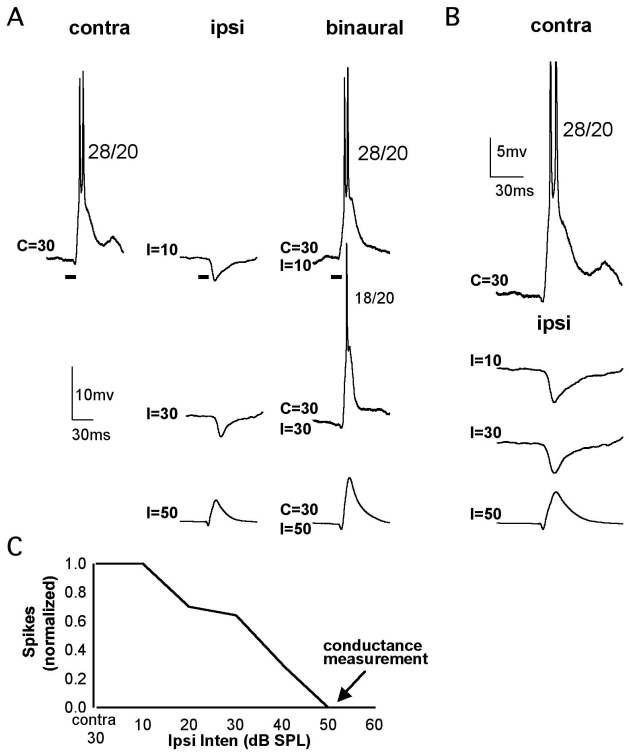

Fig 3.8. Responses of an EI cell in which ipsilateral signals evoked IPSPs at low intensities and EPSPs at high intensities. **A~C:** same with Fig 3.5.

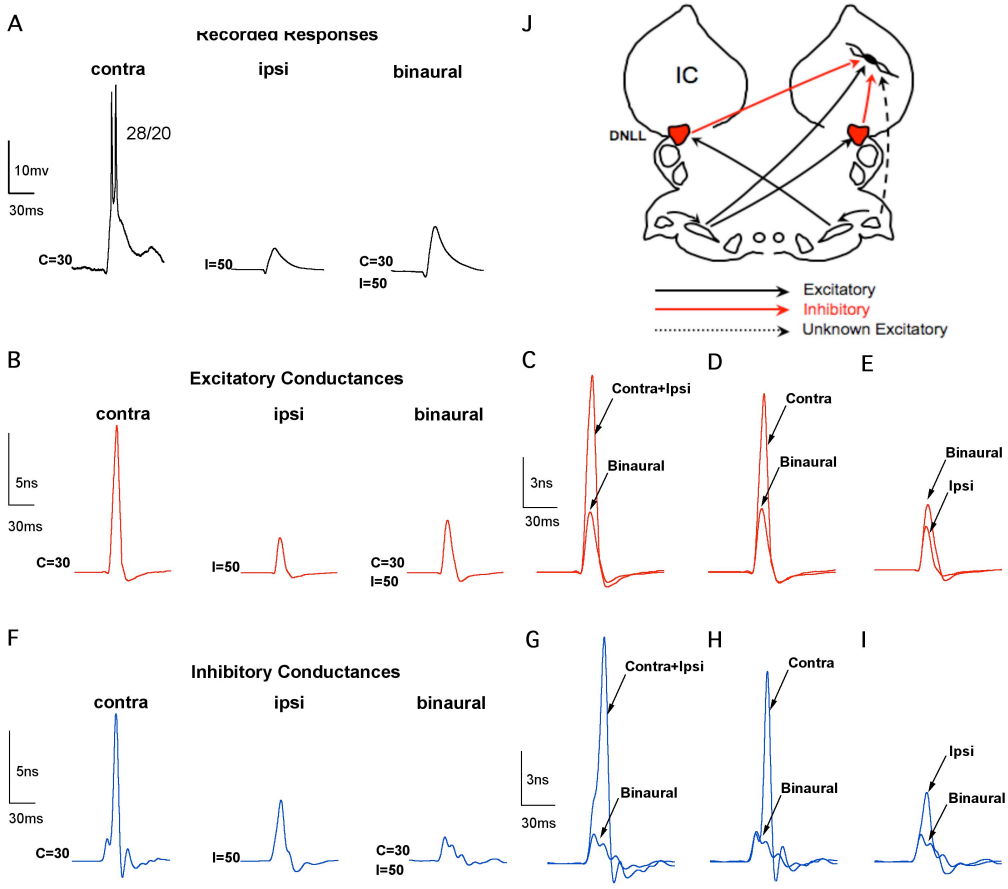

Fig 3.9. Conductances derived from the EI cell in Fig 3.7. **A~J:** same with Fig 3.6.

Conductances evoked in EI/f cells

In the previous chapter, I showed that EI/f cells are different than conventional EI cells in that facilitated spike-counts were evoked over a small range of IIDs with low ipsilateral intensities. Since spike-counts with binaural tones were enhanced above those evoked by the contralateral signal alone, it was not surprising that the same ipsilateral intensities that evoked facilitation when presented binaurally, evoked a small EPSP when presented alone. I also showed that ipsilateral signals at higher intensities evoked increasingly larger EPSPs, as did ipsilateral signals in most conventional EI cells, and the same ipsilateral signals presented binaurally, suppressed contralaterally evoked discharges, features that also occurred in conventional EI cells.

Based on the PSPs evoked by monaural and binaural tones, the same circuitry proposed for the EI cells with ipsilateral EPSPs was proposed for the EI/f cells that expressed ipsilateral EPSPs at the IID that evoked facilitation. Specifically, two projections could account for the PSPs and spikes, one from the LSO that actually generated the EI property, and a second excitatory projection that is driven by the ipsilateral ear (Fig 2.8C). The only difference in the EI/f cells is that the circuit that generates the ipsilateral EPSP has a lower threshold than the ipsilateral excitatory circuit in the conventional EI cells. This circuitry predicts that binaural signals with low ipsilateral intensities should evoke both the excitation from the LSO and a small ipsilaterally evoked EPSP whose summation would generate a spike-count greater than

that evoked only by the LSO excitation, and thereby evoke the facilitation. Binaural signals with higher ipsilateral intensities progressively inhibit the LSO and at the same time evoke a progressively larger excitation via the ipsilateral projection. The reason that spikes are suppressed at the IC is that the suppression of excitation at the LSO is greater than the increase in ipsilateral excitation, which is always subthreshold. In this way, binaural signals with larger IIDs, with stronger ipsilateral intensities, generate a progressively larger EPSP at the IC, due to the increase in the subthreshold ipsilateral excitation, while simultaneously suppressing discharges at the IC as a consequence of the even larger reduction in the excitatory drive from the LSO.

I next tested this hypothesis by deriving the underlying conductances evoked by monaural and binaural stimulation from the EI/f cell in Fig 3.10. Conductances were derived at two IIDs. One IID, C=10dB SPL, I=10dB SPL, evoked facilitation, while the other IID, Contra=10dB SPL, Ipsi=30dB SPL, evoked a subthreshold EPSP but also suppressed all spikes. The above hypothesis, based on the evoked PSPs, only has excitatory conductances and predicts both the monaurally and binaurally evoked excitatory conductances evoked at the two IIDs (Fig 3.10C). Specifically, the prediction is that there should be a linear summation of contra- and ipsilaterally evoked excitatory conductances at IID that evoked facilitation. The prediction for the higher IID, which evoked spike suppression, is that the excitatory conductance evoked by binaural stimulation should be substantially smaller than the linear summation of the contra- and ipsilaterally evoked excitatory conductances due to the reduction excitatory input from

the LSO. Below I first consider the conductances evoked at the IID that evoked facilitation and show that they only partially support the hypothesis based on the PSPs. I then turn to the conductances evoked at the higher IID.

Conductances evoked by the IID that evoked facilitation

The first point to be made is that tones presented to the contralateral and ipsilateral ear evoked both excitatory and inhibitory conductances (Fig 3.11B, E), and thus the hypothesis presented above is oversimplified. The excitatory conductances were basically in agreement with the prediction but the inhibitory conductances, especially the ipsilateral inhibitory conductance, were unexpected since there was no evidence of IPSPs in the PSP responses.

The excitatory conductance evoked by the contralateral tone at 10 dB SPL was large and a smaller excitatory conductance was evoked by the ipsilateral tone at 10 dB SPL (Fig 3.11B). The summation of the two monaural conductances was equal to the excitatory conductance evoked by tones presented binaurally at the same intensities (Fig 3.11C). Thus the behavior of the excitatory conductances is in agreement with the prediction presented above. That is, a linear summation of the excitatory inputs evoked by each ear does generate a facilitation. The problem is that the excitatory conductance evoked by the contralateral tone is very large and predicts a PSP far above threshold and should evoke a much higher spike count than the 10 dB SPL tone actually evoked, which

was only 2 spikes/10 stimuli. It is in this regard that the inhibitory conductances are significant.

As shown in Fig 3.10E, the inhibitory conductance evoked by contralateral tone at 10 dB SPL was large and suppressed the response that would have been evoked by the excitatory conductance alone, thereby accounting for the weak discharge evoked by the contralateral tone. The ipsilateral tone also evoked an inhibitory conductance, but one that was very small. The summation of the contra- and ipsilaterally evoked inhibitory conductances was just slightly larger than the binaurally evoked inhibitory conductance both in terms of peak amplitude and width (Fig 3.11F). What this shows is that the facilitation was generated by the summation of excitatory conductances and the facilitated response evoked by the binaural signal was then scaled down by inhibition, especially by the contralaterally evoked inhibition.

The slight difference between the contra- and and binaural inhibitory conductances (Fig 3.11G) suggests that the inhibitory conductances was also reduced by a very small degree by binaural stimulation and hence both excitatory and inhibitory inputs originated from a lower binaural nucleus. The binaural nature of the contralateral inhibitory conductance is confirmed by the conductances evoked at the higher IID, as discussed below.

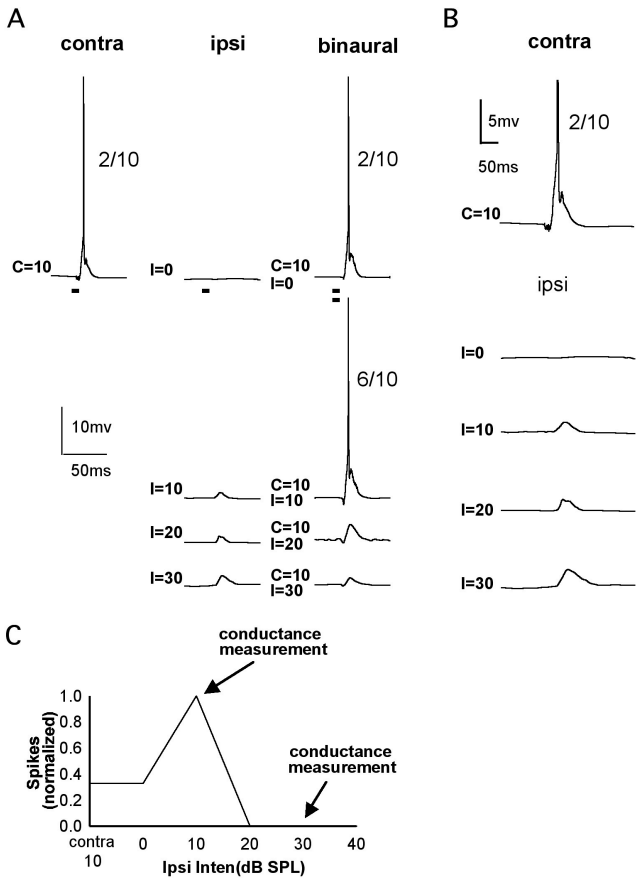

Fig 3.10. Responses of an EI/f cell. **A:** An EI/f cell in which ipsilateral signals evoked EPSPs. **B:** Excitatory response to a 10 dB SPL contralateral tone aligned with EPSPs evoked by ipsilateral tones at different intensities showing that the latencies of the EPSPs were coincident with the response evoked by a contralateral tone. **C:** IID function. The arrows showed two intensities at which the conductance measurements were done. One IID produced a binaural facilitation, and the other IID produced a binaural suppression.

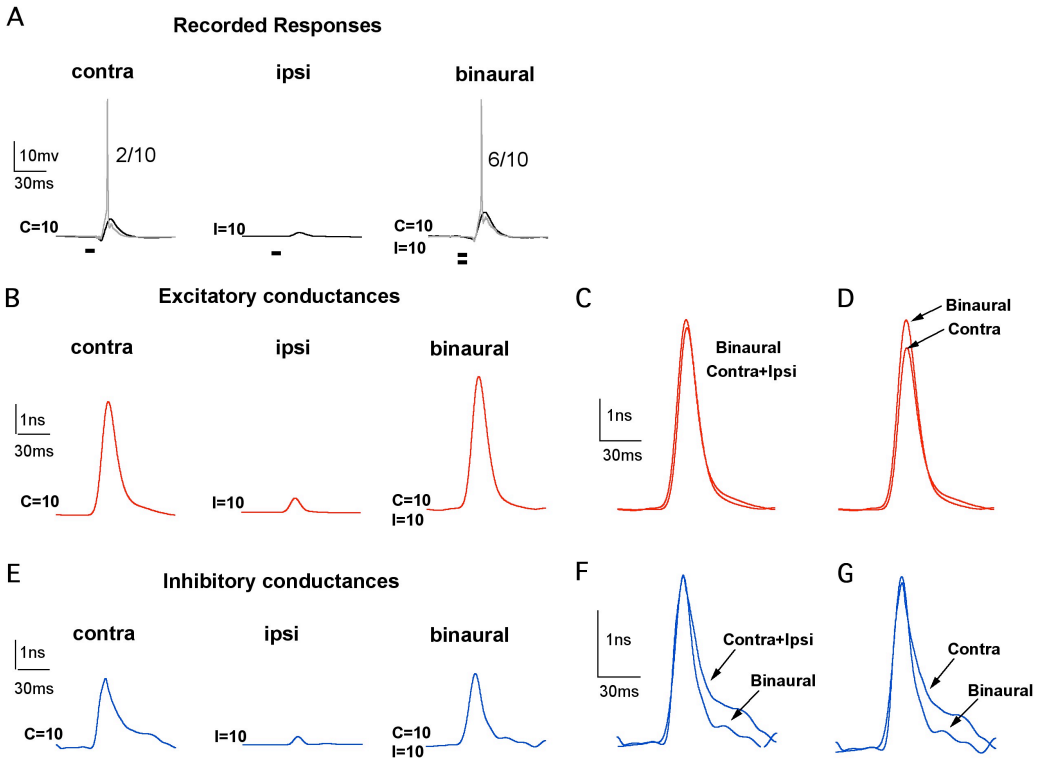

Fig 3.11. Derived conductances at the IID which showed a binaural facilitation. **A:** Recorded PSPs and spikes with a contralateral tone at 30dB SPL, an ipsilateral tone at 50dB SPL, and binaural tones (Contra=30dB SPL and Ipsi=50dB SPL). **B:** Excitatory conductances derived. **C:** A comparison between binaural excitatory conductance and the summation of contra- and ipsilateral excitatory conductances. **D:** A comparison between binaural and contralateral excitatory conductance. **E:** Inhibitory conductances derived. **F:** A comparison between binaural inhibitory conductance and the summation of contra- and ipsilateral inhibitory conductances. **G:** A comparison between binaural and contralateral inhibitory conductance.

Conductances evoked at the IID that evoked spike suppression

Conductances at the higher IID, C=10dB SPL, I=30dB SPL, showed that the mechanism for spike suppression at large IIDs in EI/f cells (Fig 3.12) is basically same as that in the conventional EI cells with ipsilaterally evoked EPSPs (Fig 3.7). The conductances suggest that the spike suppression at high IIDs is due to a complete suppression of the LSO as a consequence of the strong ipsilateral signal, and thus no contralateral excitation is conveyed to the IC. At the same time, the inhibitory innervation evoked by contralateral stimulation is also completely suppressed by the ipsilateral tone at the ipsilateral DNLL. Consequently, binaural stimulation with high IIDs completely suppresses all excitatory and inhibitory inputs that are evoked by monaural stimulation of the contralateral ear. The only response left with binaural stimulation is the response evoked by the monaural excitatory and inhibitory inputs evoked by stimulation of the ipsilateral ear.

The reasoning for proposing this circuitry can be best understood by first comparing the excitatory and inhibitory conductances evoked by the ipsilateral ear with the binaural conductances, which are shown in Fig 3.12B~I. Notice that the ipsilateral and binaural excitatory conductances are virtually the same (Fig 3.12E), suggesting that the ipsilaterally evoked excitation was not influenced by stimulation of the contralateral ear and thus originated from a monaural nucleus. I also point out that while the waveforms of the ipsilateral and binaural inhibitory conductances are slightly different

(Fig 3.12I), their magnitudes (area under the waveform) are similar. I suggest that the reason for the difference in waveform shapes is simply due to random variations that occurred on different trials. If so, then the ipsilateral inhibitory conductance is also not influenced by stimulation of the contralateral ear and it, like the excitation, originates from a lower monaural nucleus. The monaural origin of the ipsilateral inhibition is supported by the nearly identical PSPs evoked by the ipsilateral tone at 50 dB SPL and the binaural tone when the contralateral tone was 10 dB and the ipsilateral tone was 50 dB SPL. Additionally, the PSPs evoked by ipsilateral and binaural tones were virtually the same even when the contralateral intensity was increased from 10 to 30 dB SPL (data not shown). The ipsilaterally evoked PSP was generated by the ipsilateral excitatory and inhibitory conductances, and since the ipsilateral PSP was unaffected by 10 or 30 dB tones at the contralateral ear, the conductances evoked by ipsilateral tones must have originated from lower monaural nuclei.

The arguments presented above provide a strong support for the hypothesis that the response evoked by the binaural tone at the higher IID was evoked only by the excitatory and inhibitory circuits driven by the ipsilateral ear. What, then, happened to the substantial excitatory and inhibitory conductances evoked by contralateral tones? The answer, as described previously, is that both the excitation and inhibition evoked by contralateral tones originated from lower binaural nuclei, i.e., the LSO and the DNLL, and the relatively strong ipsilateral tone inhibited the activity in both lower nuclei. The contralateral excitatory conductance, for example, is large yet the binaural excitatory

conductance is equal to the ipsilateral excitatory conductance. This shows that all of the binaural excitatory conductance can be attributed to the excitatory conductance evoked only by the ipsilateral ear; there was no excitatory conductance evoked by the contralateral ear because it was completely inhibited at the LSO. The same argument is applicable to the inhibitory conductance; the binaural inhibitory conductance is much smaller than the contralaterally evoked inhibitory conductance, but has the same magnitude as the ipsilateral inhibitory conductance. Thus, the ipsilateral inhibitory conductance alone can account for the binaural conductance because the contralateral inhibition was suppressed in the DNLL by the strong ipsilateral signal.

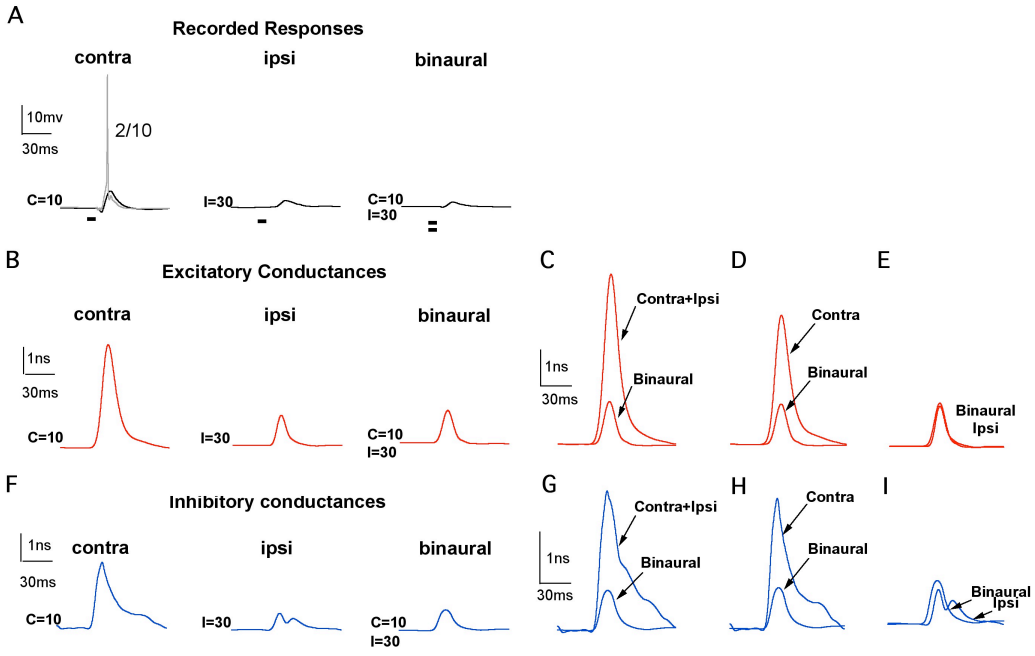

Fig 3.12. Derived conductances at the IID which showed a binaural suppression. **A~I:** same with Fig 3.6.

Summary for EI/f cells

The behavior of the conductances and PSPs described above led me to propose the following working model to explain the response features of EI/f cells. With low IIDs, the ipsilateral signal is weak and evokes a weak excitation and a weak inhibition. At the same time, the contralateral tone, although it also is weak, evokes a stronger excitation and inhibition. The excitatory inputs from both ears sum to generate the facilitation, but the inhibition evoked by both tones scales the excitation so that the spike count evoked by the binaural signal is larger than the spike count evoked by the contralateral signal alone, but still much lower than the spike count that would have been evoked only by the summed excitatory inputs. With stronger ipsilateral tones (higher IIDs) the stronger ipsilateral tone inhibits both the LSO and DNLL, and thus quenches both the excitatory and inhibitory inputs evoked by the contralateral ear. However, the stronger ipsilateral signal also evokes both excitation and inhibition from monaural nuclei and their summation generates is the only response at the IC. The net response generated by the ipsilateral inputs is a subthreshold EPSP that is virtually identical to the EPSP evoked a tone presented only to the ipsilateral ear at the same intensity as the ipsilateral tone in the binaural stimulus. Thus, the response evoked by binaural tones with a high IID is a complete suppression of contralaterally evoked spikes coupled with a subthreshold EPSP evoked by the monaural projections activated by the ipsilateral tone.

In summary, at the smaller intensity difference, the excitatory inputs driven by the contralateral simulation and ipsilateral stimulation are both monaural, they interact linearly at the IC. A binaural stimulation drives the excitatory inputs on both sides, thus evoking a bigger response. However, the inhibitory inputs driven by the contralateral simulation are binaural, and they are inhibited by ipsilateral stimulation at lower nuclei. Therefore less inhibitory inputs were evoked by binaural stimulation. Both increasing of excitation and reduction of inhibition caused by the binaural stimulation contributes to the facilitation of responses.

DISCUSSION

This study of conductance analysis showed 4 major results of EI and EI/f cells in the IC. The first is that in every cell from which we derived synaptic conductance, the circuit is far more complicated than what were predicted by extracellular recordings and PSPs. Most of the EI cells not only receive binaural excitatory innervations from LSO, but also receive binaural inhibitory innervations from the same side of the DNLL. Second, the EI property can be inherited at least partially from LSO even though the ipsilateral tones evoked IPSPs across different intensities. This result confirmed that circuits inferred only from PSPs might be inconclusive. Third, in the EI cells in which ipsilateral stimulation evoked EPSPs, both excitatory and inhibitory inputs driven by the

contralateral stimulation are binaural. Therefore our predictions based on PSPs were oversimplified. Forth, as we expected, ipsilateral EPSPs are important in shaping the binaural facilitation in EI/f cells.

Ipsilateral responses are independent of binaural property

My results have shown that binaural property could be accurately predicted based on the response evoked by monaural stimulation of contralateral and ipsilateral ear. I have shown that spike suppression in response to binaural stimulation happened in all EI cells, but these EI cells responded to the ipsilateral stimulation in different ways. A few cells have IPSPs in response to ipsilateral stimulation. A few showed no response in response to ipsilateral stimulation. Many cells show a mix of IPSPs and EPSPs evoked by ipsilateral stimulation. While the other cells showed only EPSPs in response to ipsilateral stimulation, which are totally counter intuitive. Because if an ipsilateral stimulation evoked excitation, then bigger response should be expected in response to the binaural stimulation. Therefore, we can conclude, the binaural property of a cell should not be predicted only based on the interactions between the responses evoked by the contralateral and ipsilateral signals.

Then what determines the binaural property of an IC neuron? My conductance analysis has shown that the binaural property was mostly inherited from inputs themselves. I have shown that in most EI cells, the inputs, both excitatory and inhibitory

inputs driven by the contralateral stimulation are actually binaural. The binaural excitatory inputs are most likely from LSO and the binaural inhibitory inputs are most likely from DNLL which is at the same side of the IC. When the binaural signal is present, both LSO and DNLL get suppressed, less excitation and less inhibition is evoked. Excitation has a bigger impact on the PSP due to its larger driving force. Therefore the binaural stimulation produces a small PSP, and result in a spike suppression if the PSP is below the spike threshold.

Circuits revealed by conductances are more complicated than what were predicted by extracellular studies and PSPs

The circuits of an EI cell in the IC predicted by PSPs and extracellular studies have various combinations of 4 projections; 1) the projection of the LSO to the contralateral IC; 2) the projections of the DNLL to the contralateral IC; 3) an excitatory projection of unknown source from a lower monaural nucleus that was activated by stimulation of the ear contralateral to the IC; and 4) a circuit for ipsilateral evoked EPSPs. However, conductances showed that the circuits are far more complicated and surely have more components. In the EI cells in which ipsilateral stimulation evoked IPSPs, we predicted that the excitation evoked by the contralateral stimulation interacts with the inhibition evoked by the ipsilateral stimulation locally in the IC, therefore producing a spike suppression with binaural signal. However, conductances showed the contralateral

excitatory inputs are binaural, presumably from LSO. Therefore, the binaural property in this cell is at least partially inherited from LSO. In the EI cells in which ipsilateral stimulation evoked EPSPs, we predicted that the binaural property is inherited from LSO, with additional excitatory inputs driven by the ipsilateral stimulation. However, conductances showed that not only the excitatory inputs are binaural, but the inhibitory inputs driven by contralateral signal are also binaural. These binaural inhibitory inputs were never predicted by any previous study. In the EI/f cells, we predicted that at low ipsilateral intensities, the binaural facilitation is due to the excitation evoked by the ipsilateral stimulation, at higher ipsilateral intensities, the binaural suppression is caused by the suppression of LSO. Conductances are partially consistent with the predictions. It is consistent in that at low ipsilateral intensity, contralateral excitation summed with the ipsilateral excitation at the IC, therefore a binaural signal evoked a larger excitation. It is not consistent in that at higher ipsilateral intensities, a binaural signal suppressed excitatory and inhibitory binaural nuclei, thus evoking a smaller response, a mechanism same with most EI cells which were described before.

EI and EI/f cells may share the same circuit

Previous studies suggested that EI/f cells have different circuits than EI cells. EI/f cells receive excitatory innervations from the opposite LSO, and also receive inhibitory projections from the ipsilateral DNLL. However, our results showed that most EI cells

share the same circuit with EI/f cells. How the same circuit produces different binaural property? My explanation is that the binaural property is determined by the difference in in the threshold of DNLL and LSO. This is how it works. In both EI cells and EI/f cells, binaural stimulation with a very low ipsilateral intensity evoked both excitation from the opposite LSO and inhibition from the ipsilateral DNLL. The inhibition from the DNLL suppressed the excitation from the LSO, thereby producing the low spike counts in the IC cell at IIDs that favored the contralateral ear. If the ipsilateral DNLL cell is inhibited at a lower ipsilateral intensity than the LSO cell, when the ipsilateral intensity increases and generates a bigger IID, the DNLL is inhibited but the LSO is not. Therefore an increased spike count is evoked at the IC cell because the excitation from the LSO is unopposed by DNLL inhibition. This is how the facilitation is produced in EI/f cells. But if the ipsilateral DNLL cell is inhibited at a higher or same ipsilateral intensity than the LSO cell, when the ipsilateral intensity increases and generates a bigger IID, the LSO is inhibited, or both LSO and DNLL are inhibited, thus evoking a smaller spike count. This can explain the binaural suppression in EI cells. At yet higher ipsilateral intensities, both DNLL and LSO cells are suppressed thereby reducing and then completely eliminating any discharges from the IC cell. This is true for both EI and EI/f cells.

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